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GENERAL BIOLOGY OF THE PROTOZOAN LIFE CYCLE[1]

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FOR five decades after the time of Ehrenberg, the peculiar conception of a protozoan as a miniature replica of a metazoan, held by this gifted observer, influenced the study of Protozoa. This influence gradually wore off and, so far as morphology is concerned, ended with the careful observations of Stein, Claparède and Lachmann, Engelmann, Bütschli and Hertwig, who showed that various structures of the protozoan body are not beating hearts, brains, ovaries and stomachs, but are simple differentiations of the single-celled organisms.

A more lasting influence of Ehrenberg's teaching, seen even to-day, is the habit of regarding a single protozoon as the complete expression of a species equivalent to an individual worm, mollusc or mammal. The individual metazoon dies, while the protozoon does not die but grows to full size and divides into two or more—facts which led Weismann to his conclusions regarding mortality in Metazoa and immortality in Protozoa.

We owe to Maupas the credit for dissipating this last reminiscence of Ehrenberg's teaching, and for showing that the single cell is not the final representative of a protozoon species. We are accustomed to the idea that many

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individuals of a polymorphic coelenterate are present in potential in the fertilized egg of the coelenterate, but we are less accustomed to the idea that polymorphic individuals are present in potential, in the fertilized cell of a protozoon. Research in recent years has shown that successive generations of Protozoa may be more or less progressively differentiated, so that a cell picked out at one phase of the life cycle is quite a different type of individual from one picked out at another phase. Which, for example, would be the "type" individual of the dimorphic Foraminifera? Which would be the type in the reproducing flagellated and ameboid stages of *Nägleria punctata?* of different phases in the life history of *Centropyxis, Arcella,* or *Difflugia?* or of intestinal and blood-dwelling stages of *Plasmodium?* The morphological differences here indicate that the protozoan life history involves differentiation analogous to that of a polymorphic metazoon, and justify the comparison of the whole life cycle with the development and differentiation of a metazoon, especially that of a metagenetic type such as coelenterate or trematode.

The importance of the whole life cycle, first demonstrated by Maupas, was fully recognized by Schaudinn and applied by him to the study of parasitic forms. The monographs resulting from this study, especially those on *Coccidium schubergi, Plasmodium vivax* and on rhizopods, are classics in the literature of Protozoa, and models which later students have followed.

Through Schaudinn's work, and by later researches, the sequence of events in different parasitic types has been made out with painstaking care until to-day, we know the general history of the majority of injurious human protozoan parasites, the modes of transmission from host to host, the types of intermediate hosts and what happens in them. In short, we know enough to furnish an adequate basis for public and private prophylaxis which, in the hands of sanitary commissioners and public health officers, has put an end to epidemics of yel-

low fever, malaria and dysentery; has rehabilitated vast tracts of land in Italy; saved millions of dollars in South Africa and in our southern states, and has made the Panama Canal possible.

Such are the first, and practically the most important, results of our knowledge concerning protozoan life cycles; quite enough, indeed, to justify the science of Protozoology. Important as these results are, we are not at all satisfied; we know too little about the conditions of development; too little about the nature of the vital processes of the organisms themselves and their variations in structure and function under differing conditions, ignorance which must be cleared away before much further practical advance can be made. Further advance will be less spectacular and must be based upon the biological study of the organisms as units of protoplasmic substance, and this will rest upon working hypotheses supported by experiment. It is along such theoretical lines that I wish to direct your attention for a few minutes, to develop a conception of the life cycle as a whole, and to offer a theoretical interpretation of the different phases of vitality and of structural variations.

Let us consider for a moment, a single *Ameba* or a malaria germ, not as a cause of disease, but as a unit mass of protoplasm which, like a free-living *Paramecium* or *Didinium,* performs all of the fundamental vital activities common to living things, namely nutrition, excretion, irritability and reproduction. The chemical composition of these unit masses, so far as I know, has never been made out, but there is no reason to doubt that it agrees with that of other living substances, since the accompanying properties of protoplasm—metabolism, growth and reproduction—are obviously performed, and probably in the same way. In such unit masses of protoplasm we assume that processes of hydrolysis, synthesis, oxidation and reduction, are constantly going on as in other protoplasms, and not in any haphazard way, but always orderly and under regulative control of the organism as a whole.

The appearance of *Ameba* shows that the protoplasm is made up of alveoli and inter-alveolar substances of different density, representing colloidal and crystalloidal substances in a general mixture which Ostwald describes as an emulsoid. Between these different substances constant chemical activities are in progress, and the orderliness which distinguishes these processes in the protoplasm of the living organism from similar processes which go on in the same protoplasm when crushed, are possibly due, as Mathews states, to the physical barriers of cellular and nuclear membranes, alveoli, and the colloidal centers of activity. The speed with which such processes take place in living protoplasm, which, in itself, distinguishes living processes from chemical processes in lifeless substances, is due to specific enzymes or catalyzers which are manufactured as a result of chemical activities in living protoplasm. These bring about and control each successive step in the long chain of chemical actions involved in destructive metabolism, the action in each event being conditioned by the nature of the protoplasmic substratum. In this chain of destructive processes different substances may be formed which undergo no further oxidation or other chemical change, but are stored up in the protoplasm until disposed of by excretion, these products, leading to changes in the protoplasmic substratum, *i. e.,* to protoplasmic chemical differentiation, may or may not be accompanied by visible structural differentiations. Such products of destructive metabolism, in the form, usually, of nucleo-proteins or their derivatives, may act as poisons to other organisms, as melanin does to the host in malaria, or as the proteolytic ferments of *Entameba histolytica* do in dysentery; or they may play some important part in the vital activities of the organism itself, as in phosphorescence of *Noctiluca* and the dinoflagellates, or more generally, in regeneration and reproduction.

Let me illustrate this latter point by some experiments made on *Uronychia transfuga,* a ciliated protozoon. This organism has rather a complicated structure with nine

giant cirri at the posterior end (Fig. 1). Under laboratory conditions it divides once a day approximately, or, more exactly, once in twenty-six hours. The first indication of division is the precocious formation of the giant cirri in a central region of the body which we have called the "division zone." The experiments were undertaken for the purpose of studying the relative power of regeneration of the single cell at different ages between divisions, it having first been determined that the cell regenerates readily after being cut. Cells were cut with a scalpel at different periods subsequent to division; some during the end stages of division; some 15 minutes after division; some one hour after; others 2, 4, 8, 12, 16 and 20 hours after, and some were cut just prior to the next division period, *i. e.*, 24 to 25 hours after division. In all cases of record, the cells were so cut that one portion contained the micronucleus and part of the macronucleus, the other portion containing only a part of the macronucleus. The former, or, as I shall call it, the nucleated portion, invariably regenerated after some hours, forming a perfect cell, the latter, without a micronucleus which I shall call the enucleated portion, behaved differently as regards regeneration, according to the age of the cell when cut. In all cases this portion lived from three to five days after the operation. If the recently divided cell were cut at any period up to 16 hours after division the result was the same; no regeneration occurred, the fragment merely rounded out, swimming about by its adoral membranelles (Fig. 2, 3). If the cells were cut when from 18 to 24 hours old, regeneration occurred not only in the nucleated portion, but in the *enucleated fragment as well*, the percentage of regeneration increasing with the increased age of the cells when cut, until at the age of 24–25 hours the enucleated fragments regenerated perfectly in 100 per cent. of cases (Fig. 4, 5, 6, 7).

These results indicate a gradual chemical differentiation of the protoplasm as a result, probably, of destructive and constructive metabolic processes. The giant

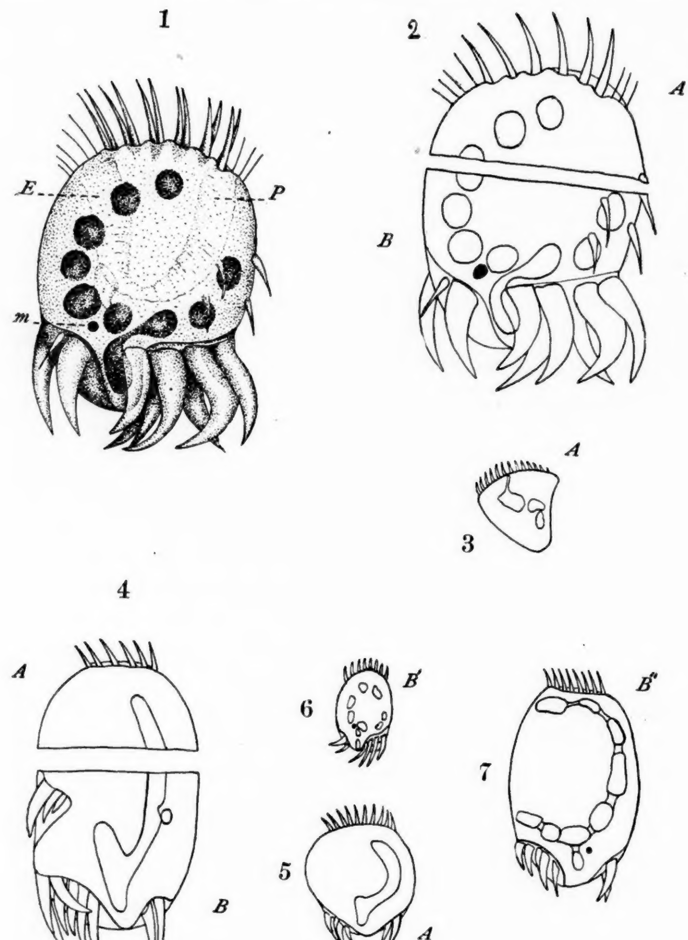

FIG. 1. Normal adult individual of *Uronychia transfuga* with macronucleus in the form of large chromatin spherules; micronucleus (*m*); endoral membrane (*E*); pre-oral membrane (*P*); and large posterior cirri.

FIGS. 2 AND 3. Individual 12 hours old cut as shown in 2. Part *A* had no micronucleus and after 72 hours appeared as shown in 3 *A*. Part *B* regenerated perfectly in 24 hours.

FIGS. 4, 5, 6 AND 7. Individual cut at age of 25 hours as shown in 4. *A* regenerated perfectly, except for absence of micronucleus, in 24 hours (5 *A*); *B* divided through the original division plane (indicated in 4), within a few hours forming a minute but perfect individual (6B′), and a normal full-size individual (7 *B*″).

cirri which are regenerated are the visible expression of inherited structures characteristic of the species. Since the enucleated fragment from a cell cut when young does not regenerate while the nucleated fragment does, we must conclude that one essential factor at least, necessary for the production of these inherited structures, lies in the micronucleus.

The giant cirri, furthermore, are visible differentiations which are precociously formed at division. This must mean that the inherited factors find their expression at this period, and it follows from the successful formation of giant cirri in enucleate fragments from old cells, that whatever may be the direct causative agent or agents in the process they must be generally distributed throughout the protoplasm at this time. We have no direct evidence as to what these agents may be; possibly there is only one and that of the nature of a specific enzyme, or perhaps some chemical body analogous to hormones formed as a result of mutual interaction of nucleus and cytoplasm when the latter has reached a certain stage of chemical differentiation through normal activities. Or it is possible that such chemical bodies are present at all times and are activated only when the protoplasmic substratum reaches some particular stage of development. Thus it is possible that, with continued metabolism, the acidity of the protoplasm gradually increases until a concentration is reached in which specific enzymes, not able to act before, are now activated.

However theoretical the interpretation of the phenomenon may be, the periodic and temporary power of regeneration is an observed fact indicating a difference in the protoplasmic make-up at different age periods, a difference which may be satisfactorily expressed by the phrase cumulative chemical differentiation.

Another observed fact is that the regenerative power is exhausted with cell division, for young enucleated fragments do not regenerate. This indicates a reduction of the differentiated adult protoplasm to the condition of young cells; or, at least, the protoplasm is restored to a

state where the causes underlying regeneration are inactive. This may be due to the exhaustion of specific substances which take part in the reaction of regeneration, or it may be due to the chemical and physical changes accompanying cell division.

We are led through these experiments, to further speculations concerning the nature of cell division. Chemical differentiation of the protoplasm continues even after the stage is reached when regeneration is possible. This is shown by the fact that formation of the cirri in *Uronychia* precedes the process of division in normal cells, and by the additional fact that regeneration of cirri occurs while cell division does not occur in enucleate fragments cut from old cells. I would interpret cell division as due to cytolytic action set up by enzymes or other chemical bodies produced as a result of interaction of nucleus and cell body differentiated chemically by age. Cytolysis may then occur more or less extensively throughout the entire protoplasmic mass, but it is most active in the division zone of the organism which is more highly differentiated than other regions (see Calkins, 1911, and Peebles, 1912). The membrane of the cell turns in at this cytolyzed division zone and the constriction results in cell division.

As a consequence of the activities accompanying cell division the protoplasmic substratum is reduced from the differentiated adult condition to the condition characteristic of young cells, and the processes of growth and chemical differentiation, division and de-differentiation, recur in more or less rhythmical succession.

Viewing the life cycle as a whole, there are two phases which must be taken into account. These are, first, the encystment phase, and second, the sexual or conjugation phase, both widespread and almost universal in protozoan life histories. Let us first consider the encystment phase.

Encystment occurs ordinarily when the conditions in the surrounding medium are adverse, such as desiccation, lack of food, etc., such encysted forms emerging from the cyst when suitable conditions are restored. In some cases also, encystment occurs during the digestion of food. In

addition to these casual encystments there is another form of encystment which involves more deeply-lying activities of the protoplasm. In *Didinium nasutum* I have found that encystment occurs at periodic intervals which cannot in any way be connected with adverse conditions of the environment or with feeding, but must be interpreted as a normal phenomenon due to internal conditions of the organisms. Encystment at such times persists for from 5 to 8 days and during this period no amount of coaxing will bring the organisms out. During such encystment the macronucleus fragments into hundreds of small chromatin particles which are ultimately absorbed in the cytoplasm; the micronuclei divide, and products of their division give rise to a new macronucleus and new micronuclei. When the process is completed and the organisms emerge from their cysts they possess from five to seven times the vitality, as measured by the division rate, of the same race prior to encystment. Fermor was the first in 1913 to describe similar happenings during the encystment of *Stylonychia;* in this case, dissolution of the old macronucleus and absorption of the fragments, fusion of the two micronuclei and formation of new macronuclei and micronuclei from the fusion nucleus, were described.

It is well known that *Paramecium* does not encyst. Nevertheless Woodruff and Erdmann (1914) have shown that phenomena similar to those occurring during encystment in *Stylonychia* and *Didinium,* and which they refer to under the general term "endomixis," recur at periodic intervals (about once a month) in the case of *Paramecium aurelia.* Here also the old macronucleus fragments and the fragments are absorbed in the cytoplasm, while a new macronucleus and micronuclei are formed from the division products of the old micronuclei.

The interpretation of this set of phenomena in the life history of protozoa is a perplexing problem. There is not a doubt that vitality, as measured by the division rate, is restored. Likewise there is little reasonable doubt that a complete chemical and physical reorganization of the protoplasm takes place. The renewal of vitality was shown

both in Woodruff's culture and in my *Didinium* culture, and one general problem is stated in the query: how long can such periods of reorganization continue? Woodruff believes that they may keep on indefinitely, but in my experiments with *Didinium* the race apparently lost its power to encyst and ultimately died out after six months' culture without encystment. So too, in my culture of *Paramecium caudatum* (1902) where similar reorganization occurred at least twice, the race ultimately lost the power to reorganize and died out. I may have had unfavorable forms to start with and so lost both races at early dates. It is interesting in this connection, however, to note that Whitney, working with the rotifer *Hydatina*, a metazoon, carried a race through nearly 200 generations by parthenogenesis when the individuals lost their power to reproduce in this way, and many of his lines died, while others produced sexual individuals.

The general biological effect of this process of reorganization is a new chemical combination with a new potential of metabolic activity, and a new lease of life. Not only are the nuclei restored to activity, but the cytoplasm is likewise completely reorganized by the distribution through it of relatively large quantities of nucleo-proteins, giving rise to successive derivatives (through hydrolysis, oxidation, reduction, etc.), all increasing the metabolic processes and releasing more chemical energy expressed by activity of movement and feeding, and leading to more rapid assimilation and growth, all indicated by an increased division rate. In short, the protoplasm is rejuvenated.

The second phase in the life history to be considered, viz., the sexual phase, involves still more deeply-reaching protoplasmic activities. The protoplasm of the individual cells at this period has a different physical, and presumably chemical, make-up than during ordinary vegetative periods. In free-living forms, such as the ciliates, the outer protoplasm becomes sticky or glutinous so that two cells on touching, fuse together. In this condition which I have called the "miscible state" conjugation is possible, and the physical condition may be so extreme that groups

of cells get stuck together. I have witnessed the fusion of nine *Paramecium caudatum* cells in a single amorphous mass.

In other forms, notably the parasitic protozoa, protoplasmic changes at this stage follow two lines of differentiation. Some cells store up metabolic products in the form of reserves of nutriment and develop into female gametocytes or macrogametes. Others develop into more active male gametocytes and microgametes. In both of these differentiated types if union or fertilization is prevented, the cells die a natural death.

The effects of conjugation or fertilization are almost the same as those following asexual reorganization through encystment. In ciliates cytolysis of the old macronucleus takes place and its substances are absorbed, that is, undergo chemical changes in the cytoplasm. The majority of the maturation nuclei, both in free-living and in parasitic forms, meet the same fate, while a new nuclear apparatus results from the products of the fertilization nucleus or synkaryon. The cytoplasm is renewed in a chemical sense and metabolic activities recommence with renewed vigor; a new race is started. The sole difference from encystment is that reorganization occurs after or during amphimixis and a new hereditary complex is formed in the nucleus, while even this, in endogamous conjugation at least, can not be very different from the condition after asexual reorganization. It is obvious that, if conjugation is the equivalent of fertilization in metazoa, asexual reorganization or endomixis is the equivalent of parthenogenesis.

What is the significance of these two important phases in the life cycle and how can they be interpreted in terms of metabolic activities? As we have seen, there is reason to believe that the cell protoplasm becomes progressively differentiated in a chemical sense between division periods, until just prior to division processes take place which do not occur at earlier periods. With division this differentiated condition is reduced, possibly through cytolysis, until a more labile protoplasm results. Now it is not at

all improbable that such reducing processes are more or less incomplete, so that the protoplasmic substratum in the second generation is different from that of the first. We have evidence of this in the foraminifera where differences in the protoplasmic structure and in shell structure characterize the second generation. Further evidence is seen in the rhizopods, where increasing quantities of chromidia, and in some cases differences in shell structure, are morphological indications of differentiation.

Furthermore, it is not improbable that such differences are cumulative from generation to generation, just as chemical differentiation is cumulative with inter-divisional age, until a protoplasmic substratum is evolved in which processes not possible before can now take place. We have shown that *Paramecium* at the conjugation phase has a different physical make-up than at other times, the cortical plasm becomes mucilaginous and fusion results on contact, while physiological differences are manifested by the invariably decreasing division rate during and after this period when conjugation is possible. Here the protoplasmic substratum is differentiated, and processes occur which are not possible at other times. So, too, in *Didinium*, *Stylonychia*, etc., with successive generations a protoplasmic substratum is gradually evolved (possibly hastened by adverse conditions) in which the peripheral zone of protoplasm undergoes cytolysis and forms an impervious membrane—the cyst membrane—analogous to the fertilization membranes of metazoan eggs. Further cytolytic changes, involving hydrolysis, reduction and other chemical activities, are set up in the cell body, especially in the cell nuclei which divide or fragment. As a result of these activities, which are more profound than those accompanying cell division, the protoplasm is again restored to a labile condition, vitality is renewed and a de-differentiated protoplasm begins a new cycle of metabolic and reproductive phases.

The phenomena of conjugation may be interpreted in a similar way as due to processes possible only in a substratum produced by cumulative protoplasmic differentia-

tion. A visible expression of such differentiation is seen again in the chromidia formation of *Sarcodina* and in the dimorphic gametocytes of foraminifera and Sporozoa. The reorganization phenomena are quite as complicated and as far reaching as after encystment, and the end result is the same, a de-differentiated protoplasm and a new individual with a high potential of vitality. If fertilization is prevented the differentiated macro- and microgametes die as do metazoan eggs and spermatozoa, and a similar result follows the continued culture of free-living ciliates in which conjugation, or its equivalent, asexual endomixis, is prevented.

In all life histories we find more or less regular cycles of vegetative and sexual phases, complicated by more or less active asexual and sexual reproduction. In parasitic forms it is possible, I may say probable, that reorganization and renewal of vitality take place during encysted stages as Schaudinn, Wenyon and others have held for the genus *Entameba;* or, as in *Paramecium,* they may take place without encystment in types like *Plasmodium* as described by Schaudinn. The processes of autogamy, so-called, described for different types of *Entameba,* may be interpreted as asexual endomixis, and the conflicting views as to the significance of nuclear structures in *Entameba coli, E. histolytica, E. tetragena* and *E. minuta,* may all be reconciled when this possibility of asexual reorganization is applied to the various parasitic rhizopods.

With *Plasmodium,* the principle of asexual reorganization and renewal of vitality, or parthenogenesis, has long been called upon to explain malaria relapse. The process, as described by Schaudinn, is too familiar to need repetition here. Despite the objections which have been raised in recent years against this interpretation, it must be admitted that no *à priori* difficulty stands in its way. It is evident from experiments that the protoplasm of an old race is more stabile than that of a young race, possibly due to accumulation of products of metabolism in the former, either for a useful purpose, as in the storage of yolk material in a female cell, or for some harmful purpose, as in

Paramecium caudatum during depression. In either case if a labile protoplasm can be restored resulting in chemical activities which ultimately bring about dissolution of these formed products, then renewed vitality is the outcome. Asexual reorganization effects this result, but the same result was produced artificially by the use of salts in my experiments with *Paramecium caudatum* during conditions of depression, and in cases where the cell body was visibly loaded with products which it could not automatically dispose of. The splendid results which Bass has obtained in cultivating *Plasmodium* in vitro and in the presence of sugar, indicate the possibility of malaria organisms while in a stabile condition being similarly changed into a labile condition by changes in the blood content of the host. Changes thus set up might well be the equivalent of asexual reorganization or parthenogenesis, or the equivalent of fertilization in restoring vitality.

In this sketch of the protozoan life cycle I have endeavored to give a comprehensive though somewhat speculative account of the different phases of vitality which may apply equally well to any type of Protozoa. Cell division, reorganizing encystment or its equivalent, and conjugation, are all regarded as phenomena of the same general character but differing in degree, the effect in each step being the restoration of the protoplasm to a condition more or less free from cumulative metabolic differentiations.

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THE EVOLUTION OF THE CELL. III

BY THE LATE PROFESSOR E. A. MINCHIN, F.R.S.

In the phase of evolution that I have termed the pseudomoneral or cytodal phase, in which the organism was a droplet of periplasm containing scattered biococci or chromidiosomes, metabolism would result in an increase in the size of the cytode-body as a whole, accompanied by multiplication of the chromidiosomes. Individualization of the cytodes would tend to the acquisition of a specific size, that is to say, to a limitation of the growth, with the result that when certain maximum dimensions were attained the whole cytode would divide into two or more smaller masses amongst which the chromidiosomes would be partitioned.

In the next stage of evolution, the protocyte with a definite nucleus, it is highly probable that at each division of the cell-body, whether into two or more parts, the primitive method of division of the nucleus was that which I have termed elsewhere "chromidial fragmentation";[26] that is to say, the nucleus broke up and became resolved into a clump of chromidiosomes, which separated into daughter-clumps from which the daughter-nuclei were reconstituted. Instances of nuclear divisions by chromidial fragmentation are of common occurrence among the Protozoa and represent probably the most primitive and direct mode of nuclear division.

It is clear, however, that if the chromatin-grains are to be credited with specific individuality and qualitative differences amongst themselves, this method of nuclear division presents grave imperfections and disadvantages, since even the quantitative partition of the chromatin is inexact, while the qualitative partition is entirely fortuitious. Chromidiosomes having certain specific properties might all become accumulated in one daughter-cell,

[26] *Op. cit.*, p. 101.

and those having opposite properties in the other, so that the two daughter-cells would then differ entirely in their properties.

I can but refer briefly here in passing to the interesting theory put forward by Bütschli, to the effect that sexual phenomena owe their first origin to differences between cellular organisms resulting from the imperfections of the primitive methods of cell-division. If we assume, for instance, as so many have done, that one of the earliest qualitative differences between different chromatin-granules was that while some influenced more especially the trophic activities of the cell, others were concerned specially with kinetic functions; then it might easily happen, after nuclear division by chromidial fragmentation, that all, or the majority of, the kinetic elements pass into one of the two daughter-cells, while its twin-sister obtains an undue preponderance of trophic chromatin. As a consequence, some cells would show strong kinetic but feeble trophic energies and others the opposite condition, and in either case the viability of the cells would be considerably impaired, perhaps inhibited. If it be further assumed that cells of opposite tendencies, kinetic and trophic, attract one another, it is easy to see that the union and fusion of two such cells, the one unduly kinetic (male) in character, the other with a corresponding trophic (female) bias, would restore equilibrium and produce a normal cell with kinetic and trophic functions equally balanced. On this view, sexual union, at its first appearance, was a natural remedy for the disadvantages arising from imperfect methods of nuclear division.

It is not surprising, therefore, to find that the process of nuclear division undergoes a progressive elaboration of mechanism which has the result of ensuring that the twin sister-granules of chromatin produced by division of a single granule shall be distributed between the two daughter-cells, so that for every chromatin-grain obtained by one daughter-cell an exact counterpart is obtained by the other; in other words, of ensuring an exact qualita-

tive, as well as quantitative, partition of the chromatin-particles. In its perfect form this type of nuclear division is known as karyokinesis or mitosis, and all stages in its progressive development are to be found in the Protozoa.

In the evolution of nuclear division by karyokinesis two distinct processes are being developed and perfected in a parallel manner, but more or less independently; first, the method of the partition and distribution of the chromatin-grains between the two daughter-nuclei; secondly, the mechanism whereby the actual division of the nucleus and the separation of the two daughter-nuclei are effected in the cell-division. I have dealt elsewhere[27] with the evolution of the mechanism of karyokinesis as exemplified by the numerous and varied types of the process found amongst the Protozoa, and I need not discuss the matter further here, but the behavior of the chromatin-grains may be dealt with briefly. The main feature in the process of the exact quantitative and qualitative distribution of the daughter-chromatin between the daughter-nuclei is the aggregation of the chromatin-grains or chromioles into definite, highly individualized structures known as chromosomes. In the most perfected forms of the process of chromosome-formation the chromioles become united into a linear series termed by Vejdovský a chromoneme, which is supported upon a non-chromatinic basis or axis. According to Vejdovský, the supporting substance consists of linin; R. Hertwig, however, in his well-known studies on *Actinosphærium*[28] considers that the supporting and cementing substance of the chromosome is plastin derived from the substance of the nucleoli. However that may be, the essential feature of the chromosome is the cementing together of the chromioles to form the chromoneme, a thread of chromatin which may be disposed in various ways on the supporting axis, sometimes being wound spirally round it (Vejdovský).

[27] *Op. cit.*, pp. 105–120.
[28] *Abhandl. bayer. Akad.* (II. Cl.), XIX, 1898.

The actual division of the chromatin takes place by the longitudinal splitting of the chromoneme, in other words, by simultaneous division into two of each of the chromioles of which the thread is composed. In this way every chromiole which was contained in the original chromoneme is represented by a daughter-chromiole in each of the two daughter-chromonemes. It follows that the familiar process of the splitting of the chromosomes in karyokinesis is a mechanism which brings about in the most simple, sure and direct manner an exact quantitative and qualitative partition of the chromatin-grains between the two daughter-nuclei. In the sequel each daughter-nucleus is built up, according to Vejdovský, entirely and solely from one of the two daughter-clumps of chromosomes, and each chromosome is resolved again into its constituent chromioles, giving rise in some cases to a definite portion of the nucleus, a karyomere, from which again, at the next nuclear division, the chromosome is reconstituted by the chromioles falling into line in an orderly manner.

The chromatin-cycle of a cell in which the process of division by karyokinesis takes place in its most perfectly developed form, may, therefore, be conceived as follows: The nucleus in its resting state contains a definite number of companies or brigades of chromatinic units (chromioles), each brigade spread over a certain extent of the nuclear framework forming a karyomere. As a preparation to division each separate brigade of chromioles falls into line as the chromoneme, forming with its supporting substance the chromosome; there are formed, therefore, just so many chromosomes as there were karyomeres in the nucleus. In this disciplined and orderly array each chromiole undergoes its division into two daughter-chromioles, so that each file or chromoneme of chromioles splits into two files. At the reconstitution of the daughter-nuclei each daughter-chromosome gives rise to a karyomere again, the chromioles falling out of the ranks and disposing themselves in an apparently irregular manner on the

newly-built framework of the daughter-nucleus to constitute their own particular karyomere. Thus karyokinesis differs only from the most primitive method of division by chromidial fragmentation in that what was originally a haphazard method of distribution has become a disciplined and orderly manœuvre, performed with the precision of the parade-ground, but in a space far less than that of a nutshell.

In the nuclear division of Protozoa, without going into excessive detail, it may be stated broadly that all stages are to be found of the gradual evolution of the tactical problem which constitutes karyokinesis. The chromosomes in the more primitive types of nuclear division are usually very numerous, small, irregular in number and variable in size; the splitting of the chromosomes is often irregular and not always definitely longitudinal; and distinct karyomeres have not so far been recognized in the nuclei of Protozoa. In many cases only a part, if any, of the chromatin falls in to form the chromosomes, and a greater or less amount of it remains in the karyosome, which divides directly into two. The various types of nuclear division in Protozoa have been classified as promitosis, mesomitosis and metamitosis, for detailed accounts of which those interested must refer to the textbooks and original descriptions.

I have dealt briefly with the problem of the evolution of karyokinesis because the process of nuclear division is, in my opinion, of enormous importance in the general evolution of living organisms. I have expressed elsewhere[29] the opinion that the very existence of multicellular organisms composed of definite tissues is impossible until the process of karyokinesis has been established and perfected. For tissue-formation it is essential that all the cells which build up any given tissue should be similar, practically to the point of identity, in their qualities; and if it is the chromatin-elements of the cell which determine its qualities and behavior, then the exact qualitative

[29] *Op. cit.*, p. 120.

division of the chromatin, as effected in karyokinesis is indispensable as a preliminary to the production of identically-similar daughter-cells by division of a parent-cell. Hence it becomes intelligible why, amongst Metazoa, we find the occurrence of nuclear division by karyokinesis in its most perfect form to be the rule, and "direct" division of the nucleus to be the rare exception, while, on the other hand, in the Protista, and especially in the Protozoa, we find every possible stage in the gradual evolution of the exact partition of the chromatin in the process of nuclear division, from chromidial fragmentation or the most typical amitosis up to processes of karyokinesis as perfect as those of the Metazoa.

There now remains only one point of general interest in the evolution of the cell to which brief reference must be made, namely, the divergence of animal and vegetable cells. Not being a botanist, I desire to approach this question with all caution; but as a protozoologist it seems to me clearly indicated that the typical green plant-cell took origin amongst the Flagellata, in that some members of this group of Protozoa acquired the peculiar chromatophores which enabled them to abandon the holozoic or animal mode of life in exchange for a vegetative mode of nutrition by means of chlorophyll-corpuscles. It is well known that many of these creatures combine the possession of chlorophyll with an open, functional mouth and digestive vacuoles, and can live either in the manner of plants or of animals indifferently or as determined by circumstances. It would be interesting to know exactly what these chromatophores, at their first appearance, represent; whether they are true cell-organs, or whether, as some authorities have suggested, they originated as symbiotic intruding organisms, primitively independent. I do not feel competent to discuss this problem. I would only remark here, that if the green plant-cell first arose amongst the Flagellata, then the distinction between plant and animal (that is, green plant and animal) is not so fundamental a divergence in the series of living beings

as is popularly supposed, but is one which did not come into being until the evolution of organisms had reached a relatively advanced stage, that, namely, of the true nucleated cell.

I have confined myself in this address to the evolution of the cell as this organism is seen in its typical form in the bodies of the multicellular organisms, starting from the simplest conceivable type of living being, so far as present knowledge enables us to conceive it. But there is not the slightest reason to suppose that the evolution of the Protista took place only in the direction of the typical cell of the cytologist. Besides the main current leading up to the typical cell there were certainly other currents tending in other directions and leading to types of structure very unlike the cells composing the bodies of multicellular organisms. It is impossible that I should do more here than indicate some of the divergent lines of evolution, and I will confine myself to those seen in the Protozoa.

Taking as the starting-point and simplest condition in the Protozoa a simple cell or protocyte, in which the body consists of a small mass of cytoplasm containing a nucleus, with or without chromidia in addition, an early specialization of this must have been what I may term the plasmodial condition, typical of Rhizopods in which the cytoplasm increased enormously to form relatively large masses. The nucleus meanwhile either remains single and grows very large or, more usually, a great number of nuclei of moderate size are formed. From this large plasmodial type is to be derived the foraminiferal type, characterized by the creeping habit of life, and probably also the radiolarian type, specialized for the floating pelagic habit. Both foraminiferal and radiolarian types are characterized by an excessive development and elaboration of skeletal structures, and the geological record proves that these two types of organisms attained to a high degree of specialization and diversity

of form and structure at a very early period.[30] The Mycetozoa exemplify another development of the creeping plasmodial type adapted to a semi-terrestrial mode of life.

In the Mastigophora the body generally remains small, while developing organs of locomotion and food-capture in the form of the characteristic flagella. In this class there is a strong tendency to colony-formation brought about by incomplete separation of sister-individuals produced in the ordinary process of reproduction by binary fission. The so-called colonies (they would better be termed families) show a most significant tendency to individualization, often accompanied by physiological and morphological specialization of the component flagellate individuals.

As an offshoot, probably, from ancestors of the Mastigophoran type arose the Infusoria, the Ciliata and their allies, representing by far the most highly organized unicellular type of living being. No cell in the bodies of the Metazoa attains to such a complication of structure as that exhibited by many Ciliates. In the Metazoa the individual cells may be highly specialized for some particular function of life; but a Ciliate is a complete and independent organism and is specialized for each and all of the vital functions performed by the Metazoan body as a whole. From the physiological standpoint a Ciliate (or any other Protist) is equivalent and analogous to a complete Metazoon, say a man, but I can not for a moment agree with Dobell[31] that the body of a Ciliate is homologous with that of a Metazoon—not at least if the word homologous be used in its usual biological sense of homogenetic as opposed to homoplastic. Dobell appears to me to negative his own conclusion when he maintains that the body of a Ciliate is "non-cellular" while admitting that the Metazoon is multicellular; how then can they be said to be homologous? Only if the term homologous be

[30] For Foraminifera see especially Heron-Allen, *Phil. Trans.* (B), Vol. 206 (1915), p. 229.

[31] *Journal of Genetics*, IV (1914), p. 136.

used in a sense quite different from its ordinary significance.[32]

In addition to the highly developed structural differentiation of the body the Infusoria exhibit the extreme of specialization of the nuclear apparatus in that they possess, as a rule, two distinct kinds of nuclei, micronuclei and macronuclei, composed respectively of generative and trophic chromatin, as already pointed out. This feature is, however, but the culminating point in a process of functional specialization of the chromatin which can be observed in many Protozoa of other classes, and which, moreover, is not found invariably in its complete form in all Ciliata.

In this address I have set forth my conceptions of the nature of the simplest forms of life and of the course taken by the earliest stages of evolution, striving all through to treat the problem from a strictly objective standpoint, and avoiding as far as possible the purely speculative and metaphysical questions which beset like pitfalls the path of those who attack the problem of life and vitalism. I have, therefore, refrained as far as possible from discussing such indefinable abstractions as "living substance" or "life," phrases to which no clear meaning can be attached.

How far my personal ideas may correspond to objective truth I could not, of course, pretend to judge. It may be that the mental pictures which I have attempted to draw are to be assigned, on the most charitable interpretation, to the realm of poetry, as defined by the greatest of poets, rather than of science.

> The lunatic, the lover and the poet
> Are of imagination all compact;
>
> . . . .
>
> And as imagination bodies forth
> The forms of things unknown, the poet's pen
> Turns them to shapes and gives to airy nothings
> A local habitation and a name.

If I might be permitted to attempt an impartial criticism of my own scheme, I think it might be claimed that

[32] See Appendix A.

the various forms and types of organisms in my evolutionary series, namely, the simple cell or protocyte, the cytode or pseudomoneral stage, the micrococcus, even the biococcus, are founded on concrete evidence and can be regarded as types actually existent in the present or past. On the other hand the *rôle* assigned by me to each type in the pageant of evolution is naturally open to dispute. For example, I agree with those who derive the Bacteria as primitive, truly non-cellular organisms, directly from the biococcus through an ancestral form, and not at all with those who would regard the Bacteria as degenerate or highly-specialized cells. But the crux of my scheme is the homology postulated between the biococcus and the chromatinic particle—chromidiosome or chromiole—of true cells. In support of this view, of which I am not the originator, I have set forth the reasons which have convinced me that the extraordinary powers and activities exhibited by the chromatin in ordinary cells are such as can only be explained on the hypothesis that the ultimate chromatinic units are to be regarded as independent living beings, as much so as the cells composing the bodies of multicellular organisms; and, so far as I am concerned, I must leave the matter to the judgment of my fellow-biologists.

I may point out in conclusion that general discussions of this kind may be useful in other ways than as attempts to discover truth or as a striving towards a verity which is indefinable and perhaps unattainable. Even if my scheme of evolution be but a midsummer-night's fantasy, I claim for it that it coordinates a number of isolated and scattered phenomena into an orderly, and, I think, intelligible sequence, and exhibits them in a relationship which at least enables the mind to obtain a perspective and comprehensive view of them. Rival theories will be more, or less, useful than mine, according as they succeed in correlating more, or fewer, of the accumulated data of experience. If in this address I succeed in arousing interest and reflection, and in stimulating inquiry and controversy, it will have fulfilled its purpose.

Appendix A.—The Cell-Theory

The most recent attack on the Cell-theory, as it is understood by the majority of modern biologists, has been made by Mr. Dobell, who, if I understand him rightly, refuses to admit any homology between the individual Protistan organism and a single cell of the many that build up the body of a Metazoon. On the contrary, he insists that the Protist is to be regarded as homologous with the Metazoan individual as a whole. On these grounds he objects to Protista being termed "unicellular" and insists that the term "non-cellular" should be applied to them.

As regards the cellular nature of the Protista, it is one of my aims in this address to show that amongst the Protista all stages of the evolution of the cell are to be found, from primitive forms in which the body can not be termed a cell without depriving the term "cell" of all definable meaning, up to forms of complex structure in which all the characteristic features of a true cell are fully developed. Thus in the Protozoa we find the protoplasmic body differentiated into nucleus and cytoplasm; the nucleus in many cases with a structure comparable in every detail to that of the nucleus of an ordinary body-cell in the Metazoa; reproduction taking place by division of the body after a karyokinetic nuclear division often quite as complicated as that seen in the cells of the Metazoa and entirely similar both in method and in detail; and in the sexual process of differentiation of the gametes on lines precisely similar to those universal in Metazoa, often just as pronounced, and preceded also in a great many cases by phenomena of chromatin-reduction comparable in principle, and even sometimes in detail, with the reduction-process occurring in Metazoa. I really feel at a loss to conceive what further criteria of homology between a Protozoon and a Metazoan cell could be demanded by even the most captious critic. On the ground of these and many other similarities in structure and behavior between the entire organism in the Protozoa and the individual cell, whether tissue-cell or germ-cell, in the Metazoa, the case

seems to me overwhelmingly convincing for regarding them as truly—that is to say, genetically—homologous.

Looking at the matter from another point of view, namely, from the standpoint of the Metazoa, it is true that in the groups of most complicated and highly organized structure the cells often develop secondary connections or fusions due to incomplete division, to such an extent that in parts of the body the individuality of the primitively distinct cells may be indicated only by the nuclei (as may occur also in Protozoa, for example, in associated gregarines); but in all Metazoa certain of the cells retain permanently their complete independence and freedom of movement and action. In the Metazoa possessing the simplest and most primitive types or organization, such as sponges and cœlenterates, the cells composing the body show far greater independence of action, and in the course of ontogeny entire groups of cells may alter their relative positions in the body as the result of migrations performed by individual cells; while it is now well known that if the adult sponge or hydroid be broken up completely into its constituent cells, those cells can come together again and build up, by their own individual activity, the regenerated body of the organism. For these reasons it seems to me impossible to regard the body-cells of the Metazoa otherwise than as individual organisms complete in themselves, primitively as independent as the individual Protozoon, and in every way comparable to it.

From the considerations summarized very briefly in the two foregoing paragraphs and capable of much greater amplification and elaboration, the view generally held that the entire organism of a Protozoon is truly homologous with a single body-cell of a Metazoon seems to me quite unassailable, and to have gained in force greatly from recent investigations upon both Protozoa and Metazoa. On the other hand, any Protist, as an organism physiologically complete in itself, is clearly analogous to the entire individual in the Metazoa—a comparison, however, which leaves the question of genetic homology quite untouched.

As regards the application of the term unicellular or non-cellular to the Protozoa, it is evident that if the evolution of living beings had never proceeded beyond the stage of the Protista, and if no multicellular organisms had ever been evolved, the term cell could then never have been invented by an intelligent being studying other living beings, supposing for an instant the possibility of such intelligence existing apart from a mammalian brain. So long as the Protozoa are studied entirely by themselves, without reference to any other forms of life, they may be termed non-cellular in the sense that they are not composed of cells. It is only when they are compared with multicellular organisms that the term unicellular becomes applicable on the ground of the homology already discussed between the Protozoon and the body-cell of the Metazoon.

THE MECHANISM OF CROSSING-OVER

II

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IV. THE MANNER OF OCCURRENCE OF CROSSING-OVER

A. Interference

As soon as it seemed probable that the factors were linked in line, and that the crossing-over was the actual method of interchange, it became of interest to discover and to analyze the precise mode of incidence of the interchange. The questions suggested themselves, for example, what was the total frequency of crossing-over, did any factors separate more often than they remained together, how often did crossing-over occur at two points simultaneously, and was there any tendency, in such cases, for the two points of crossing-over to be a definite distance apart, or in definite positions, etc. For answers to these questions might throw light on the mechanism of crossing-over, what cytological phenomena it was connected with, and what stage in synapsis it occurred at.

With these points in view the author calculated the linkage relations that would result on several possible schemes of interchange. The simplest possibility was that the chromosomes always twisted in loops of fixed length, though not of fixed position, and always underwent breakage, with recombination of homologous strands (*i. e.*, "crossed-over" in the technical sense), at each place that the strands crossed one another. In such a case there would always be a definite distance between one point of crossing-over and another; moreover, all factors which were separated by a distance great enough for double crossing-over to occur between them, *i. e.*, by the length of at least one loop, must always have either double or single (or multiple) crossing-over between them. Sturtevant's data, however, showed that this was

not true, and accordingly it had to be concluded that the length of the loop was variable, or that "crossing-over" did not always occur where the strands crossed.

Another possibility was that crossings-over were quite independent of one another, having an entirely random or chance distribution in the chromosome, with reference to each other. This would mean that when crossing-over occurred at one point, another crossing-over would be just as likely to occur coincidently at any other given point—whether this be very near or far away—as when no crossing-over took place at the first point. But this latter scheme would not be that expected on the method of crossing-over proposed by Jannsens and followed by Morgan, for in the stages when Jannsens supposed crossing-over to occur the chromosomes are rather loosely twisted, so that loops of very small length do not occur as often as longer ones (thus, very near one point of crossing-over the strands seldom cross back again). I therefore determined the mathematical relations which would exist between crossing-over frequencies, if crossings-over had a chance distribution with reference to one another, in order to compare these figures with those obtained by experiment. On the assumption that separation between A and B has no influence on separation between B and C, if crossing-over occurs between A and B in 10 per cent. of cases and between B and C in 20 per cent. of cases, then, among those ten cases in a hundred where crossing-over between A and B occurs, 20 per cent. (*i. e.*, 2 cases) would be cross-overs between B and C as well; in other words, the per cent. of double cross-overs would be equal to the product of per cents. AB and BC (formula 1). The easiest way to determine the correctness of the assumption in any given case, therefore, is to compare the observed per cent. of double cross-overs with per cent. AB $\times$ per cent. BC.

Another relation besides was found to hold between the theoretical linkage values, dependent upon the relation in formula 1. For it is easily seen that the number of *separations* between A and C must always be equal

to the sum of the number of crossings-over between A and B, and between B and C, minus all those crossings-over contained in the cases where coincidence occurred, and in which A and C, therefore, failed to separate,—*i. e.*, minus twice the number of cases of double crossing-over. Hence, if formula 1 is correct, then it must also be true that per cent. AC = per cent. AB + per cent. BC — 2 (per cent. AB × per cent. BC) (formula 2). This formula was originally expressed not only in the above terms, where the "per cent. of separations" (*i. e.*, ratio of separations to the total number of cases) is used as the index of separation frequency, but also in terms of the so-called "gametic ratio"—the ratio of cases of non-separation to those of separation—for this was the way of indicating degree of linkage then used by all investigators of the subject. The latter index gives much more complicated formulas, however, and so it was pointed out at the same time that per cent. of separations would afford a much more useful measure of linkage.

Later, Trow also worked out and published the same formula (no. 2)—in terms of the "gametic ratio"—and it is generally known as "Trow's special hypothesis" (17). But on the reduplication hypothesis held by Trow, and by the other English geneticists who do not accept the chromosome explanation, the formula would be supposed to result, not from the fact that crossing-over between A and B was independent of that between B and C, but from the fact that "reduplications" AB and BC were independent, not being disturbed by any "primary reduplication" AC. Adherents of the reduplication hypothesis have been much concerned as to whether or not their results confirmed the assumptions made in Trow's formula, and have in one or two instances calculated that they did. Let us examine for a moment the requisites for proving such a conclusion. As above shown, the whole matter turns on the frequency of coincidence of separations AB and BC (*i. e.*, on the frequency of "double crossing-over") and the question can be settled by determining directly the amount of this coincidence.

If the per cent. of double cross-overs = per cent. AB $\times$ per cent. BC (formula 1), then the assumption that separation frequencies AB and BC are independent is correct. As offspring from a back-cross all show what factors they received from the hybrid parent, a back-cross involving the three factors A, B, and C at the same time will answer the question at once, for all the cases of coincident separation (double cross-overs) that occur can be counted. But where the hybrids, instead of being back-crossed, are inbred—a practice followed by adherents of the reduplication hypothesis—then it is impossible to tell which F_2 individuals come from gametes of the classes which we may term double cross-overs, unless one of these classes is the triple recessive, and then the only double crossovers which can be known as such are those very rare individuals that happen to result from the union of two double crossover gametes. The British workers have, therefore, not been able to find the proportion of double cross-overs directly, to compare this with formula 1, but have tried to determine the frequency of coincidence indirectly, by using the method followed in formula 2. That is, they determined the relations existing between frequencies AC, AB, and BC, as calculated from their F_2 counts, for, as above shown, the greater the frequency of double crossing-over, the more will AC be cut down in proportion to AB and BC. And it seemed evident that, if the relation of AC to AB and BC was just that given by Trow's formula (2), then coincidence of separations must have the frequency demanded on the assumption that separations (or "reduplications") AB and BC occur independently of one another. As a matter of fact, however, this method offers no answer to the question, unless almost impossibly large F_2 counts are obtained, for otherwise *the independent random fluctuations of these three values in this kind of count are so great that any deviation in AC due to excess or deficiency of double crossing-over would be quite lost to sight.*

The question was, however, immediately and definitively answered in *Drosophila*, before Trow's paper ap-

peared, by examination of Sturtevant's extensive back-crosses, especially of those involving three pairs of factors at once. As the results did not conform to the formula, it was not published, but as Trow has since raised this question publicly and the adherents of the reduplication hypothesis are still discussing it, it may not be out of place to have given an analysis of it here, and to recall the fact that it had already been tried and rejected. Besides, as will appear below, a discussion of the relations which would exist if crossings-over were independent of one another is a necessary preliminary for a treatment of the relations which do exist between linkage values.

The results showed that double crossing-over does not, as a rule, occur as frequently as would be expected if, as the above formulæ assumed, it were purely a matter of chance whether or not two cross-overs happen coincidently. In a sense, then, the occurrence of one crossing-over interferes with the coincident occurrence of another crossing-over in the same pair of chromosomes, and I have accordingly termed this phenomenon "*interference.*" The amount of interference is determined by comparing the actual per cent. of double cross-overs with the per cent. expected if crossings-over were independent, *i. e.*, if they had a purely chance distribution with reference to each other. Now, the per cent. which would occur on the latter expectation has already been given by formula 1 as per cent. AB $\times$ per cent. BC. If, then, the observed per cent. of double cross-overs were divided by per cent. AB $\times$ per cent. BC, we would obtain a fraction showing what proportion of the coincidences which would have happened on pure chance really took place. This ratio of observed double cross-overs to the chance expectation appears to me to furnish the most useful measure of interference. The ratio is itself best expressed in per cent., and it may be called the relative coincidence, or simply "coincidence." If the "coincidence" is low, this means that there has been much interference, for most of the double cross-overs expected on chance were prevented from appearing; conversely, if coincidence is high, the

interference must have been very weak. Some illustrations may make the meaning of this index clearer. If, for example, coincidence is 0 per cent. no double crossing-over is occurring; the interference between one crossing-over and another is then complete. If coincidence is 45 per cent., this figure does not mean that 45 per cent. of the individuals are double crossovers, but that 45 per cent. of the number of double crossovers which would be expected as a result of pure chance (whatever that number may have been) actually appeared, 55 per cent. having been "interfered with," or somehow prevented from occurring. If coincidence is 100 per cent., there has been no interference, for the same number of double crossovers appeared as expected on the ground that the two crossings-over did not interfere with each other's occurrence. 110 per cent. would mean that if one crossing-over occurred, the other was 10 per cent. *more* likely to occur than in cases of random distributions of crossings-over. This would be "negative interference," for as coincidence increases interference decreases.

On Janssens's theory that crossing-over takes place in the strepsinema stage, when the chromosomes are twisted in loose loops, crossing-over would very seldom take place at two points very near together, for this would require a tight twisting of the chromosomes. Accordingly, on this theory interference was to be expected; furthermore it would be expected that interference was very great between crossings-over that were in neighboring regions; but between crossings-over further apart there should be little or no interference. The results were according to this expectation; they indicated strongly that the interference was very great for crossings-over short distances apart, but progressively diminished as the distances considered became greater. The conclusion drawn was that crossing-over took place as postulated on Janssens's theory, when the strands were loosely twisted in strepsinema, although the twisting and crossing-over did not take place in the stereotyped manner suggested as a first possibility, in the earlier part of this section. For there

was evidence that the distances between the two points of crossing-over in double cross-overs were variable; but this again corresponded with the fact that the chromosomes of *Batracoseps* and other forms, as seen under the microscope, did not always twist in loops of the same length. Furthermore, if it be supposed that in most maturing eggs of the fly the homologous chromosomes twist tightly enough to cross at least once or twice, as is certainly the case in *Batracoseps* and many other forms, it must be concluded that at not every point of crossing does actual "crossing-over" (recombination of strands) take place, for it was found that nearly half of the factor-groups emerged without having undergone any crossing-over at all. And this, in turn, corresponded with the observations of Janssens and others, which showed that at some at least of the points of crossing of homologous chromosomes, the latter merely untwisted again without having undergone the "chiasmatype" process. Here, then, was a theory of crossing-over that seemed complete, so far as connecting the genetic facts with the cytological observations was concerned.

B. Possible Mechanisms of Crossing-Over

There is one very unsatisfying point, however, in this original scheme of crossing-over. That is, it postulates that crossing-over occurs at a comparatively late stage in synapsis, when the strands have become very much shorter and thicker than the long delicate threads which first came into contact with their homologues (see Fig. 6). Now, in crossing-over the chromosomes must come into contact, and break, at *precisely* homologous points, otherwise factors would be lost or gained by them when crossing-over occurs. But presumably the factors are set very close together in the line, judging by the fact that mutations in new "loci" (positions in the chromosomes) are still as numerous as ever, and that, if the whole chromosome is packed with factors as close together as, judging by their linkage relations, they seem to be at certain places in it, it must contain at the very least 200 factors. It is

difficult to conceive how this cleavage of ultramicroscopic nicety can take place properly at a stage when the chromosomes are so coarse and short. The observations of Vejdovsky and others, taken in connection with the genetic results from *Drosophila,* render it practically certain that the factors are really disposed in an extremely fine,

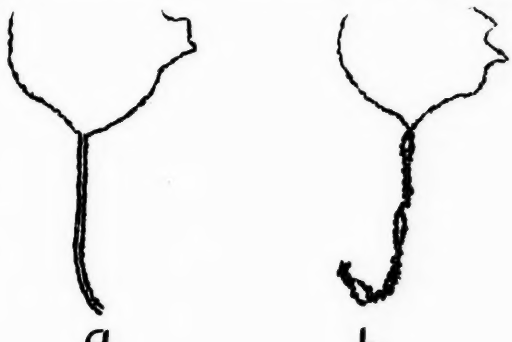

FIG. 6. Chromosomes during an early stage of synapsis (amphitene). In some preparations the apposed threads seem parallel, as in *a*; in others they seem twisted about each other, as in *b*.

long thread or "chromonema," which, during the metaphase and anaphase of mitosis, is coiled up very closely in more or less spiral fashion (probably within a viscous sheath of some sort), to form the thick dense chromosomes, but which, in the resting period and during the early stages of synapsis, becomes, to some extent at least, uncoiled and drawn out again. In this state, then, the chromosomes first pair, as shown in Fig. 6. Thus precisely homologous parts of the frail threads may become apposed to each other, so that this stage, which is called the "amphitene" stage, would seem to be the one best "adapted" for the occurrence of crossing-over. Later, when each chromosome becomes, presumably, a thick spiral, there would seem to be much greater mechanical difficulties in the way of exact apposition and breakage of parts.

On any possible theory of crossing-over, however, the known facts concerning interference should be capable of

interpretation. If crossing-over occurred during the "amphitene" stage, or not long after, would there be any possible explanation of the fact that one point of crossing-over is generally far removed from another? The explanation might be found simply in the fact that each of the "leptotene" chromosomes—*i. e.*, the finely drawn out chromosomes which are just about to undergo synapsis—pursued a general course that had few close turns in it. (For possibly it maintains the same general direction as it had when it was short and thick; the reader will recall that Boveri found that chromosomes preserve their approximate shape and position from one cell division to the next.) When, therefore, the leptotene chromosomes are being brought together by the synaptic attraction which homologous loci then bear for each other, the threads are usually crossed only at a few points, and these are generally far apart. If these initial points of crossing—which, it will be observed, have been determined by the original positions of the threads, and not by any twisting—are the points of crossing-over, interference would be accounted for, and would, in effect, be of the same general nature as on the mechanism of crossing-over postulated by Janssens.

It might at first seem hard to imagine why, on this second scheme of crossing-over, recombination—*i. e.*, "crossing-over"—should occur where the threads cross, but it should be remembered that the two threads, while coming together, often lie in about the same plane both above and below the point of crossing. If they keep to this original plane as they draw together, they will come to have the same plane of apposition just above and just below the crossing point,—although the sides of the filaments that face each other will be just the opposite in the two cases; consequently, the threads at the crossing point must undergo a very sharp twist, and if, as we must suppose, they are somewhat viscous, this may result in their breakage and recombination, or, perhaps, first in their fusion, and, later, when the pieces of the same chromosome above and below the point of crossing are wrenched

apart in opposite directions by mutual repulsion of the strands or by pulling of spindle fibres, in breakage of parts originally together. (So perhaps fusion might occur during the amphitene and breakage in the strepsinema stage; this would be a combination of schemes 1 and 2 which would account both for the exact apposition of parts and for the phenomena observed by Jannsens.) Be this as it may, at any rate, the negative argument may be given that it is just as hard to account for recombination at a later stage in synapsis as at this stage, even overlooking the objection of the thickness of the threads.

There is a serious objection to the scheme just given, however, in that, as the threads come together, they seem, in many preparations, not to keep their original plane of apposition, but to twist tightly about each other, like the strands of a rope, throughout their entire length (see Fig. 6). It is possible that the twisting of one thread about the other is merely apparent, however, and that the threads lie parallel but are simply coiling up in a spiral, in the process of forming the shorter, thicker prophase chromosomes; for, unless the spiral were very delicately preserved by the fixing agent, there would be apparent knots in it as though there were a twisting of two strands about each other. Moreover, there is evidence indicating that this tight twisting occurs only in certain species of animals. But let us assume for the moment that this very tight twisting really takes place during the amphitene stage in flies, and that crossing-over takes place at this period (this we may call scheme of crossing-over number three). Would there then be any way of explaining why one crossing-over should interfere with another near by, in view of the fact that the loops are of such small dimensions? In seeking an answer to this question, it will be helpful to bear in mind that crossing-over can be divided into just three essential processes—a bending of the chromosomes across each other, a breaking of the threads, and then a fusion of adjoining pieces (or, perhaps, the fusion of the homologous chromosomes comes first, and then the breaking of the original chromosomes at that

point). It follows from this that interference must in any case be due to one of the following three general causes: (1) Either the chromosomes are not likely to bend across each other twice at points near together (*i. e.,* the loop tends to be long), or (2) breakage at one point for some reason interferes with another breakage nearby (even though the threads are crossed at both of these points), or (3) fusion of chromosomes at one point in some way interferes with fusion of threads which are crossed in a neighboring region. That fusion at one point could interfere with fusion at another point can scarcely be imagined. And if crossing-over occurs according to scheme number three, the "loop explanation" must also be thrown out. Consequently, if crossing-over occurs at a stage of tight twisting the breakage of the threads at one point must somehow be considered to prevent another break near by. In explanation of this, breakage might be thought of as resulting from the tightness of the twisting, for then a breakage of the threads at one point would relieve the tension of the filaments for some distance along the line and so tend to prevent another breakage from occurring near by. (Later, when threads reunited at the point of breakage, pieces from homologous chromosomes would be as apt, or more apt, to lie end to end, and therefore to join, than pieces of the same chromosome. As a partial explanation of why the fragments should join again at all, it might be supposed that only the chromonemas break, the fused sheath which envelops the pair still holding the pieces together.)

It is fully realized that the above discussion is highly speculative. It is intended, however, not as a presentation of conclusions, but as a tentative suggestion of possibilities, in order to obtain some system of ideas that may furnish a temporary basis for a real attack—experimental and observational—upon the subject.

Tests for These Alternatives

Is there any way of obtaining evidence as to which of these three schemes of crossing-over is the more probable

one? Light might perhaps be thrown on the question by a closer study of interference, and it was largely for this reason that the experiment described in section V was undertaken. If, for example, interference was a result of length of loop (as would be true in schemes I and II), and the length of the loop tended to vary more or less in both directions, about a given mode, then coincidence would be relatively higher between crossings-over which were that distance apart, than between crossings-over nearer together or still further apart. In other words, as may be seen from Fig. 7, for small distances, the relative

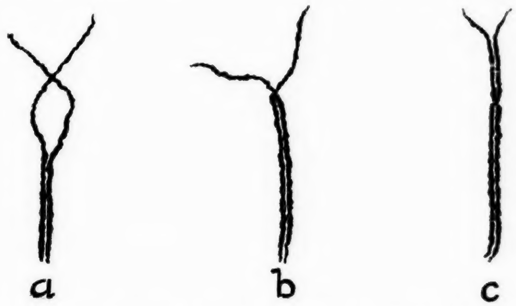

FIG. 7. Diagram to illustrate the second scheme suggested for crossing-over. The amphitene threads become sharply crossed at a particular point.

coincidence would be very small (interference high), for longer distances much greater, and with still longer distances coincidence would fall again (interference would rise). For distances double or triple the length of the loop—if the chromosomes were as long as that—coincidence would rise once more. Secondly, on the "loop explanation" of interference just outlined, coincidence should, at the modal distance, rise above the 100 per cent. level, for crossing-over would occur at a given point (K) *more* often in those cases when there is crossing-over at another point (I) lying at the modal distance from K, than in the average case. Of course it might be, however, that there was no modal length of loop—that although short loops were infrequent, all loops above a certain size were equally frequent, or that the longer the loop, the more

frequent it tended to be. In the former case coincidence would rise to a certain level, as distance between the points of crossing-over considered increased, and would after that remain constant; in the latter case it would rise progressively, and might or might not reach or pass the 100 per cent. level.

On the other hand, if crossing-over is due to a breakage of tightly twisted threads, not so many different kinds of variation of coincidence, with increase in distance, would be theoretically possible, but a condition something like the one last mentioned must always obtain. For, on scheme 3, the interference of a breakage with the tightness of twisting and consequent chance for another breakage must decrease progressively at greater and greater distances from that breakage; coincidence would thus rise until finally it reached the 100 per cent. level expected on chance. It would never rise much[2] beyond this, as one break could never make another *more* likely to occur; neither could coincidence fall once more, with a still greater distance (as it could on the loop scheme, after a "modal distance" had been reached). If, therefore, it should be found that, for certain (modal) distances between two points of crossing-over, coincidence ran well above 100 per cent., or that, beyond certain distances, coincidence fell again, there would be good evidence that crossing-over did not occur at a stage of tight twisting. If, on the contrary, it were found that crossing-over coincidence rose progressively with distance, until it reached the 100 per cent. mark, but neither went much[2] beyond

[2] Even on scheme III, coincidence could finally rise slightly above 100 per cent., for although one break (I) could not help another (K) to occur, no matter how far away the latter (K) might be, still it might, by preventing the occurrence of other breaks (J), in between these two, give more chance for the occurrence of the break farther off (K), since in this way the interference of breaks J with K (which is stronger than the interference of the more distant I with K) is removed. Thus break K might occur more often when I also occurs than in the average case, and so coincidence would rise above 100 per cent. However, it would be easy to distinguish between the slight rise in coincidence above 100 per cent., due to this cause, and the rise which would exist on the loop explanation of interference if I and K were separated by a distance about equal to the modal length. For, in the first case, *considering only gametes in which no crossing-over at all took place in between*

this, nor fell again later, and if cytological measurements should then substantiate the judgment, based on inspection, that the loops did have a modal length during the strepsinema stage, there would be good evidence that crossing-over must occur at an early stage of synapsis.

Other peculiarities of coincidence also might be found which would permit of explanation on one scheme and not on another. In groups II and III, for example, there seem to be peculiarities in the coincidence relations in cases where the chromosomes differ in regard to the factor C, or a similar factor. And a comparison of coincidence in different regions of the chromosome in any given case or in the same region of the chromosome in cases of linkage variation, might very well reveal relations that lend evidence to one scheme of crossing-over or another. Even a determination, not of coincidence, but merely of linkage variation itself, in different parts of the chromosome, might in some way shed light on the subject. In the case of the third chromosome, experiments of this sort are now under way with multiple stocks which I have made up for this purpose, and Sturtevant is conducting similar experiments with group II. The first requirement, however, is obviously an accurate study of normal coincidence, and it therefore became necessary to determine the coincidence for points various distances apart, preferably in the same experiment. But to work with a great many factors in a group at once introduced new difficulties, which made special methods necessary, as will be explained later. Before considering this experiment, it will be desirable to consider other lines of evidence and modes of attacking the problem of crossing-over.

The cytological evidence which Janssens presents for crossing-over is entirely directed towards proving that crossing-over occurs during strepsinema or later. In strepsinema the chromosomes, as already mentioned,

(*at J*), it is easily seen that the proportion of breaks at K would be lower when breakage occured at I than when there was no breakage at I, whereas in the second case, the proportion of breaks at K would in such gametes be higher when there was breakage at I than when there was no breakage at I.

become much shorter and thicker than in the amphitene stage, and each chromosome in the pair can in many preparations be seen to have split lengthwise, *i. e.*, the "tetrads" have formed preparatory to the two maturation divisions. Janssens often finds the four threads placed somewhat as shown in Fig. 8*a*, two of the threads crossing at one or two points, but otherwise being rather widely separated, and the other two threads rarely crossing but lying close to whichever one of the two threads first mentioned happens to be on the same side, and merely bending inwards and then back again where the first two threads cross. The peculiar crossing of two of the threads and the bend in the other two, as shown at point L, he interprets, in the way shown in Fig. 8*b*, as meaning

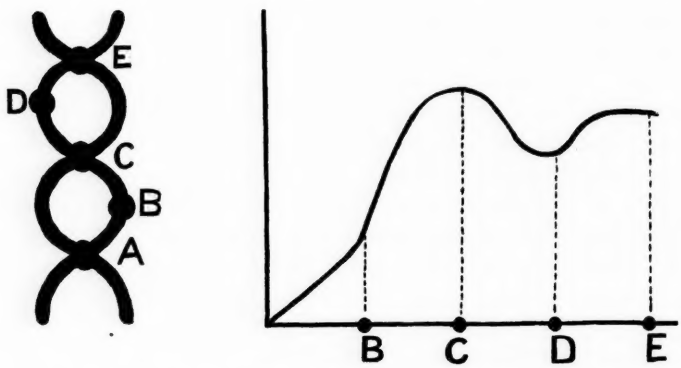

FIG. 8. Diagram to show possible coincidence relations on schemes I and II. The chromosomes are represented as crossing-over at A, and twisting in loops of the most usual (modal) length. It will be seen that a crossing-over at A will rarely coincide with one nearby—at B—since then the chromosomes would have to twist in loops much smaller than the modal length. But it will often coincide with one at C, seldom with one at D, and often again with one at E. The *relative coincidence* of crossings-over at various points on this chromosome with crossing-over at A is shown in the curve on the right.

that both pairs of threads originally were twisted across each other, but that the two homologous threads which were originally on the inner side, and so touched each other, underwent recombination, *i. e.*, "crossed over," at the point of contact; each of the new chromosomes thus formed, therefore, would lie entirely on one side or the

other; the other two threads, on the contrary, are supposed not to have undergone recombination ("crossing-over") and therefore would still lie across each other.

It would seem equally possible, however, to interpret these figures as meaning that (as shown in Fig. 9*c* and 9*d*) when the four threads began to separate into two pairs, separation happened to start at some points (A and C) between the identical halves and at other points (B) between the homologous chromosomes, it being merely a matter of chance in which way the separation started to take place. It will be seen that this would result in the formation of just such cross-figures, between two regions where separation took place in opposite ways, as Janssens finds.

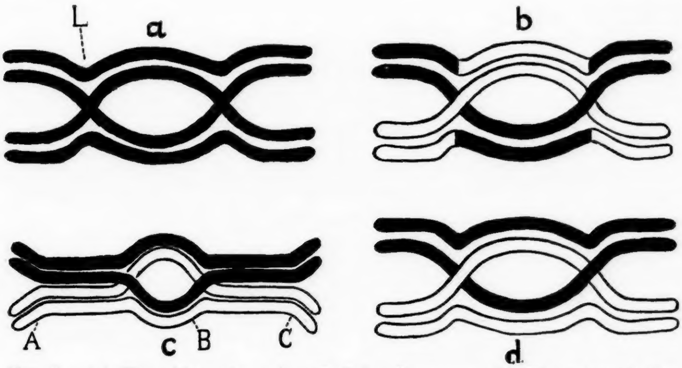

FIG. 9. (*a*) The chiasmatype described by Janssens. (*b*) His interpretation of it. (*c*) and (*d*) A suggested alternative interpretation.

Another point in Janssens's evidence is that the prophase chromosomes of maturation divisions not only show the strands crossing, at points, but often bending in towards each other near the middle, as though they had formerly crossed there, and later undergone crossing-over. It would seem possible, however, that this figure is merely due to the chromosomes remaining in contact more closely at the point where the spindle fiber is attached, and spreading apart elsewhere,—a relation which figures of Bridges and others show to exist between the two identical halves of chromosomes in the prophases of oogonial mitoses.

Finally, Janssens says that crossing-over is indicated by the fact that the chromosomes often seem to have sunken into one another at the crossing point. This detail, which would be very difficult to establish, might, of course (if it had any significance at all), merely mean that the chromosomes were still closely attached at the point where they had *previously* crossed-over. It seems incautious, therefore, to regard the cytological evidence as showing more than the possible means of the crossing-over which the evidence from factor and chromosome distribution demonstrates to occur.

Some of the Orthopteran material which gives such clear-cut chromosome figures might perhaps settle the point whether crossing-over occurs at the stage of four threads, as Janssens believed. For it is reported by Wenrich (19) that homologous chromosomes can sometimes be distinguished from one another in prophases by differences in the size or shape of contained granules, that are constant for the particular individual. Moreover, the four threads are clearly distinguishable in the prophase of the first maturation division. If a female could be found (there is some reason to believe that crossing-over does not occur in the male) which showed a difference in respect to two granules at different points in the same pair of chromosomes, then, if Janssens's theory is right, it would happen that, in some of the oocytes, of the four post-synaptic threads two would have a new combination of granules and the other two would not show any interchange. But on the view that crossing-over occurs earlier —the identical halves being formed after interchange has taken place—all four threads would be of a new combination in those cases where crossing-over of chromosomes in the region between the two pairs of granules had occurred at all.

There is an essentially similar possibility of finding out the same thing genetically. For if two threads may cross over and not the other two, then, if non-disjunction of X-chromosomes should occur in that maturation division

when the threads that crossed would normally have separated from those that did not, the egg would come to contain two X-chromosomes, one of which was a cross-over but not the other. In the usual type of non-disjunction, the X's never cross over—presumably because they paired with the Y (which was present in these cases as an extra chromosome), so this type of non-disjunction could not afford a test of the theory. But it is to be expected that non-disjunction should sometimes occur by mere accident without the interference of a Y, and since in these cases the X's could have crossed over, such cases of non-disjunction might furnish a test of Janssens's theory. In 1913, in an experiment designed for this purpose, I obtained a fly which had received two maternal X-chromosomes by reason of non-disjunction in its mother, and in which one of these X-chromosomes proved to be a cross-over but not the other! The fly resulted from a cross of a female which contained in one X-chromosome bifid and vermilion, and in the other chromosome eosin and bar, by a normal male. It itself contained in one of its chromosomes bifid and vermilion, and in the bifid, vermilion and bar. Since then Bridges has obtained other exceptions of the same general sort. But on further consideration it appears that this result really proves nothing, for the non-disjunction may just as well have taken place in an oogonial division. In this way an oocyte would result that contained three X-chromosomes. At synapsis two of these could cross over with one another, and the egg could then receive a cross-over chromosome and also an X that had not crossed over. To prove that the non-disjunction was not of this type, but really occurred in a maturation division, *i. e.*, that the two threads originated from one tetrad, it would be necessary to obtain individuals in which both of the X-chromosomes received by non-disjunction had crossed over, but each at a different point (or one of them at two points).

In a case of the latter sort the fact that both chromosomes had crossed over at some point would prove either that both of them had been in the synaptic tetrad, and so

that the non-disjunction had occurred in the maturation division in the mother fly, or else that both were derived from the two halves of a single (cross-over) X-chromosome which underwent non-disjunction in an embryonic cell division of the individual itself. But the fact that the two chromosomes are not identical would rule out the second possibility. The result, therefore, would mean that in the same tetrad one strand may have crossed over at a certain point and not another strand, *i. e.,* that Janssens's theory is correct and crossing-over takes place at a stage when there are four threads, two of which may cross over at a certain point while the others retain their original composition.

Up to the present, however, no exceptions of this type have been found, although Bridges has obtained not a few exceptions of the type that may as well be explained by non-disjunction in an oogonial division (*i. e.,* in which one X had crossed over—but not the other), and also one other exception, which had received two similar double cross-over chromosomes. The latter peculiar circumstance must have resulted either from a non-disjunction, at the maturation division in the mother, of two strands of a tetrad, both of which had crossed over in the *same two* places, or from a non-disjunction, in an embryonic cell division of the individual itself, of the two halves of the single (double cross-over) X-chromosome, which, on this view, was originally present. But the latter explanation is very improbable, for, unless the non-disjunction occurred in the first cleavage, only a small part of the fly would be composed of cells descended from the one into which the 2 X's entered; most of the cells, therefore, would contain only one X and these would necessarily be male; thus the fly would be a gynandromorph. Moreover, all the cells derived from the one which, in the non-disjunctional division, failed to receive either half of the X-chromosome, would probably die. Hence the evidence is fairly good that in this case the two double cross-over X-chromosomes represent two strands of a tetrad. Since these two strands, although both double cross-overs, were

both just alike, we must conclude either that they were both derived from the same strand, after it had already crossed over—in which case crossing-over must occur at a stage in synapsis before the homologous chromosomes split to form tetrads—or else that the tetrads were formed first, and that then crossing-over occurred at two points coincidently in the case of both pairs of threads, and at identical points in both. It is not probable, however, that, if crossing-over occurs at the stage of four threads, these two pairs of threads would both cross over at the same points, for according to the observations on which Jannsens bases the idea that crossing-over occurs at this stage, a crossing-over of both pairs of threads at the same place rarely happens. The evidence thus far gained from non-disjunction is, therefore, rather in support of the theory that crossing-over occurs at an early stage in synapsis.

D. A Case of Crossing-Over in an Embryonic Cell

It may not be out of place here to record an exceptional case of crossing-over in the male, which has not been explained. No other case of crossing-over has hitherto been found in the male Drosophila. It had been established by Altenburg and the author that the factor causing truncate wings is in the second chromosome, and further that the truncate factor is dominant under certain conditions, but it does not usually express itself unless certain intensifying factors—one in the first chromosome and one in the third—are present; even then, the character sometimes fails to develop. Thus, if a hybrid truncate male is produced by a cross of a truncate female to a black pink male (black is in chromosome II and pink in III), when this hybrid is back-crossed again to black pink females, only the gray flies will carry the factor for truncate, since in the male truncate can not cross over with the black in the homologous second chromosome. But few of the gray flies from such a cross except the gray, red-eyed females will show the truncate character, for the others will not contain both of the intensifying factors; and even in the

gray-red females the character will not always develop.

A typical count for such a cross was as follows:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
57	23	7	2	14	64	0	0	74	0	0	82
2	29	47	1	8	62	0	0	67	0	0	73

(The count of females is shown on the upper line, the count of males on the lower.) A brother of the above male, however, when similarly back-crossed, gave the following count:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
0	0	32	0	0	19	16	20	1	0	18	7
0	0	15	0	0	23	0	6	11	0	3	22

The sex-linked intensifier and the third chromosome intensifier are inherited normally as before, for the females have wings much more truncated than the males and the reds are more truncated than the pinks. But, although the truncate parent of this male contained gray in the same chromosome as truncate, and the long-winged parent contained black with long, all the truncate has crossed over, away from the gray factor and into the chromosome with black! Not a single fly has the old combination, gray truncate. It is next to impossible to imagine that the chromosomes of the second pair crossed over in the synapsis period of all the spermatocytes, and in all of them, between just these particular loci, when normally there is no crossing-over at all in the male and only 30 per cent. of crossing-over between these loci even in the female. It is, therefore, necessary to conclude that crossing-over took place once for all in a cell of the embryo, and that, as usual, it did not occur at all during spermatogenesis, although all the spermatocytes, of course, inherited the cross-over combination. It is impossible to tell whether or not the chromosomes underwent the regular process of synapsis at this early stage,

and whether they crossed over when long drawn out or when short and thick, but at least the fact remains that crossing-over may, in abnormal cases, take place in a cell before the definitive growth period is reached, and even in an individual (*Drosophila* male) in which no crossing-over is the established rule. This fact is not utterly surprising, inasmuch as even in somatic and gonial cells of Diptera homologous chromosomes show a marked tendency to lie near together (*i. e.,* to attract each other), and in Metz's preparations they may not infrequently be found even twisted about each other somewhat.

The fact that crossing-over occurs only in the female *Drosophila* is naturally of great interest, although it is of unknown significance. In the silkworms, on the other hand, Tanaka has discovered that crossing-over takes place in the male, but not in the female. Curiously enough, although these seem at first sight to be opposite cases, in both it is true that crossing-over takes place in the homozygous sex, but not in the heterozygous, for in *Drosophila* the female is homozygous for sex, the male heterozygous, and in the moth these relations are reversed. Recently, however, Castle and Wright have published data for the rat which, if sufficiently extensive, show that crossing-over happens in both sexes. The plants in which crossing-over has so far been studied have all been hermaphrodites, and crossing-over takes place in both their spermato- and oo-genesis. There is, therefore, at present no general rule which can be stated, in regard to which sex crossing-over occurs in. This fact should be taken into account in weighing the cytological evidence in regard to crossing-over, obtained in forms in which the occurrence of crossing-over has not been studied genetically. For in such cases there is always the possibility that the cytological studies are being conducted on individuals in which crossing-over does not occur and which would consequently give results quite irrelevant to the subject.

(To be continued)

FASCIATION IN MAIZE KERNELS[1]

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In the summer of 1914 a number of different varieties of corn were crossed for the purpose of studying the effect of hybridization on the weight of hybrid and pure seed produced. One of the crosses made was between Improved Leaming as the seed parent and Boone County Special as the pollen parent, the pollen of the two varieties being mixed and applied to the same ear. The former variety is a yellow dent and the latter a white dent. On this ear was found two kernels, each of which had two embryos. The description of the kernels and their progeny will be given in this paper.

Description of Kernels

In corn, the embryo is normally on the side of the kernel toward the tip of the ear. These kernels had an embryo on both sides. The kernels seemed to be normal with the exception of the extra embryo and a slight prominence or line of demarkation which extended around each kernel parallel to the embryos.

Kernel No. 1 was yellow in one half, while the other half was a paler yellow (diluted with white). Kernel No. 2 was yellow in both halves. Although there was a variation in the degree of color, the results of the F_1 generation proved that both halves of each kernel were hybrid.

Progeny from Kernels

The kernels were planted in pots in the greenhouse in April in greenhouse soil and in due time each kernel pro-

[1] Paper 2 from department of agronomy, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

FIG. 1. F_1 generation progeny of fasciated kernels. Stalks on the left, progeny of kernel No. 2, those on the right, progeny of kernel No. 1. (About one fifteenth natural size.)

duced two stalks. In May, after danger of frost was over, the contents of each pot were removed from the greenhouse and placed in the field. The time of tasseling and silking and other data were recorded during the season as shown in Table I. Each stalk produced two ear shoots; however, only one ear shoot on each stalk produced an ear.

FIG. 2. F_1 generation progeny from fasciated kernel No. 2 on the left, and fasciated kernel No. 1 on the right, showing root systems. (About one fifth natural size.)

At first the tassels and silks were bagged to prevent foreign pollination. All the pistillate flowers were self-pollinated, the pollen being applied by hand at this time. Later, paper tubes were fastened to the tassel and carried to the uppermost ear shoot, the lower ear

shoots being covered with bags and hand pollinated as was done at first.

Description of F_1 Generation Stalks

After the total growth had been made, data were recorded as to the height and diameter of stalks, length, width, and number of leaves, while the dates of tasseling and silking had been obtained previously.

Fig. 1 shows picture of entire stalks after harvesting.

TABLE I

HEIGHT AND DIAMETER OF STALKS, LENGTH AND WIDTH OF LEAVES, IN INCHES. NUMBER OF LEAVES AND DATES OF TASSELING AND SILKING OF F_1 GENERATION STALKS FROM FASCIATED KERNELS OF MAIZE

Kernel No.	Stalk No.	Number of Leaves	Height of Stalks	Length of Leaves		Width of Leaves		Diameter of Stalk	Date of	
									Tasseling	Silking
				8th	9th	8th	9th		July	
1	1	11	99	$33\frac{1}{4}$	$31\frac{7}{8}$	$3\frac{15}{16}$	$3\frac{13}{16}$	$\frac{11}{16}$	12	26
	2	13	102	$36\frac{1}{8}$	$33\frac{1}{2}$	$4\frac{1}{16}$	$3\frac{3}{4}$	$\frac{3}{4}$	16	24
2	3	14	$92\frac{1}{4}$	$36\frac{3}{4}$	$34\frac{5}{8}$	$4\frac{11}{16}$	$4\frac{5}{8}$	$1\frac{1}{8}$	12	19
	4	14	$108\frac{3}{4}$	$40\frac{1}{8}$	$37\frac{5}{8}$	$4\frac{1}{16}$	$4\frac{3}{16}$	$\frac{7}{8}$	14	21

At maturity, the entire plants were removed from the ground in such a way as to retain as many of the roots as possible. The soil was removed and a photograph was taken of the roots (Fig. 2) to especially emphasize the fact that each stalk was separate and distinct from the other and could not be classed as a tiller from the other stalk, although both were united at the radicle.

Description of F_1 Generation Kernels

Fig. 3 is a photograph of the four ears produced. All of them show Mendelian splitting. The number and ratio of yellow and white kernels will be given in Table II. None of the kernels possessed two embryos like their parents.

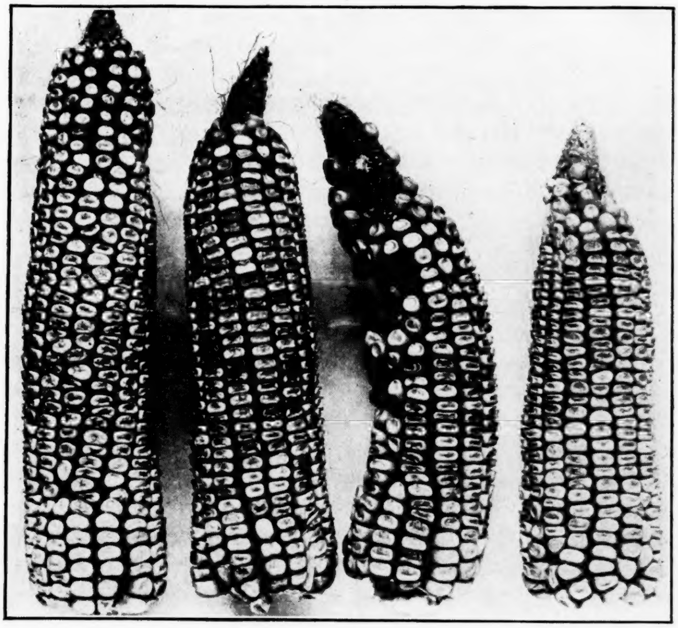


FIG. 3. F_1 generation ears produced by the fasciated kernels. Beginning at the left, the first and second ears were produced by kernel No. 2; stalks numbers 1 and 2, respectively. The third and fourth ears were produced by kernel No. 1; stalks 3 and 4, respectively. (About one half natural size.)

TABLE II

NUMBER AND RATIO OF WHITE AND YELLOW KERNELS IN THE F_1 GENERATION

Kernel No.	Stalk No.	Ear No.	Number Yellow Kernels	Number White Kernels	Ratio of Yellow to White Kernels
1	1	1	218	76	2.86:1
	2	2	377	60	6.28:1
2	3	3	393	182	2.15:1
	4	4	408	130	3.14:1
					Average ratio, 3.61:1

This generation seed will be grown next season in order to discover whether any fasciated kernels appear. After these results are obtained, a discussion of the kernels reported in this paper will be presented.

SHORTER ARTICLES AND DISCUSSION

THE INHERITANCE OF SEASONAL POLYMORPHISM IN BUTTERFLIES

ARE seasonal variations inherited, and may they play a part in evolutionary change? These are questions which Punnett in his recent book on "Mimicry in Butterflies" answers in the negative.

In no case are they known to be inherited, and in no case consequently could variation of this nature play any part in evolutionary change.

Variations to be of significance in evolution, he tells us, must be "transmissible and independent of climatic and other conditions."[1]

It would seem to require no demonstration that well-established seasonal variations like those of *Araschnia levana-prorsa* of Europe in which, it will be remembered, the ground color of the spring brood (*levana*) is red-brown, that of the summer brood (*prorsa*) black, are transmissible. Under summer conditions in Europe *prorsa* appears with the regularity of a monotypic species, true to type. Monotypic species likewise require a certain degree of temperature and amount of moisture to produce their characteristic adult coloration. *A. prorsa* is by no means peculiar in this respect. It has the definitive adult coloration of the species. That which is peculiar is the hereditary rhythmic tendency to swing from *prorsa* back to *levana*, which is so strong that experimental control can not wholly cope with it. Summer conditions artificially prolonged result in the appearance of some *prorsa* in *prorsa's* immediate offspring, but sometimes the intermediate, *porima*, is the outcome. A far larger number of individuals of the lot under experimentation, however, refuse to be forced out of the chrysalis by artificial heat, hibernate, and become *levana*. These color variations, therefore, are not subject wholly to the environment, nor wholly to heredity.

A common hereditary basis evidently underlies both of the

[1] Pp. 131, 132.

color patterns. Like produces like, but under natural conditions only by skipping a generation. Except for the innate tendency for the types to alternate, the case is similar to that of the red primrose described by Baur[2] which, growing at 15°–20° C. produces red flowers, at 30°–35° C., white. Or it is like the mutant stock of *Drosophila* described by Miss Hoge,[3] which, bred in winter or in an ice chest, gives a large proportion of flies with supernumerary legs, though in summer or in moderate temperature the stock appears to be normal. The same set of factors under varying conditions produces different results. An analysis of the factors underlying another similar case in *Drosophila*, "abnormal abdomen," has been worked out by Morgan.[4] This remarkable mutant was shown to behave as a dominant sex-linked character. It manifests itself, however, only when the food in which the flies are bred is kept moist.

The rhythmic tendency of *prorsa* to produce *levana*, notwithstanding artificial raising of the temperature, shows that this is a sort of alternation of generations in which the definitive sexual generation, *prorsa*, alternates with another apparently more primitive, *levana*, which is also sexual. This seasonal alternation of sexual forms in its hereditary basis is comparable to typical alternation of asexual and sexual types.

Weismann,[5] in discussing the case cited, assumed the presence simultaneously in the germ plasm of *prorsa*-determinants and *levana*-determinants.

> But these *prorsa*-ids were at the same time so arranged that they became active under the action of a higher temperature, if this is acting at the beginning of the pupal period, while the *levana*-ids become active at a lower temperature. Heat, therefore, is only the excitant which sets free the *prorsa*-determinants, while cold sets free the *levana*-determinants.

Modernizing Weismann's hypothesis, may we suppose that distinct Mendelian factors underlie each of these two discontinuous types of coloration? The idea is attractive, but all the evidence at hand indicates that the determinants or factors of both types are borne by all the gametes. Intermediates, showing a

2 "Einführung in die Vererbungslehre," pp. 4–6.

3 *Jour. Exper. Zool.*, 18, 1915.

4 "Mechanism of Mendelian Heredity," pp. 39–41.

5 "New Experiments on the Seasonal Dimorphism of Lepidoptera." Translation by Nicholson in the *Entomologist*, 1896.

combination of the two patterns, called *porima*, occur under certain temperature conditions. The two patterns can not be Mendelian allelomorphs of each other, though the possibility remains they may have undiscovered allelomorphs. Too little is now known of inheritance in this species for us to judge whether Weismann's hypothesis in modern form is tenable, or whether a single set of factors, or single factor, reacting differently to different environments, is sufficient to account for the two types.

In a preliminary analysis of the problem the two color phases seem like distinct ontogenetic stages. *Levana* possibly is *prorsa* with immature colors, arrested in their development through the action of cold. *Prorsa* in the chrysalis may pass rapidly through the *levana* stage into its final, complete condition. Its offspring, however, independently of the environment, hereditarily tend to hibernate in the chrysalis and become *levana*. This interpretation of the two color phases is in line with the facts of dichromatism in beetles, as described by McCracken[6] and others. *Gastroidea dissimilis*, when it emerges from the pupal case, is black, and certain individuals permanently retain this color, others, however, pass on to a permanent bright green phase. *Lina lapponica* (*Melosoma scripta*) has a spotted-brown phase which is either permanent or is replaced by black. The two color phases in each of these forms, however, are Mendelian allelomorphs of each other, the dominant color being that appearing first in ontogeny, the recessive last.

The cold weather varieties of *Colias eurytheme*, about to be discussed, certainly may be regarded as being produced in large part by the arrested development of pigmentation. In this most remarkable seasonally polymorphic butterfly of western and central North America, *Colias eurytheme*, the writer has found that the flaming orange coloration of the summer form (usually called the typical *eurytheme*) and the paler orange-yellow of the spring and autumn broods (*ariadne* and *keewaydin*) are variations also due to differences in the reaction to the environment of definite Mendelian factors. This has been shown by crossing the orange *eurytheme* of the central and western states, with the clear yellow species of the eastern and central states, *Colias philodice*, the yellow of which segregates cleanly from the orange in F_2, as a recessive. The hybrids, as well as the *eurytheme* stock, show seasonal polymorphism. The F_1 hybrids, for example, are of a

[6] *Jour. Exper. Zool.*, 3, 1906.

dilute orange. Orange is therefore incompletely dominant. The heterozygote is an intermediate. The amount of orange pigmentation, or the degree of its dilution, in the F_1 hybrids, however, varies prodigiously with the season. The summer-bred hybrid is of dilute orange ("apricot yellow") somewhat evenly distributed over the wings, but in the small winter-bred individuals, such as emerge in the greenhouse in December, the orange is restricted to a faint flush near the posterior (inner) margin of the fore wings.

But even though the underlying hereditary basis supports a superstructure that varies widely, are these variations *as such* inherited? The variety of *eurytheme* called *ariadne* is small and of a pale orange hue. This form appears under cold weather conditions only. Its dwarfness is due to the failure of the caterpillar to feed during the late fall to full size, though food is abundantly supplied. The shortness of the day evidently is a factor in checking the feeding. The caterpillar forages actively at mid-day, but becomes sluggish before nightfall, yet it matures even while it is not feeding, and hence produces a dwarfed pupa. The pale color may be readily explained by the supposition that the elaboration of chromogenic substances in the blood of the pupa is checked by the cold so that these materials ripen in the scales of the wings merely into faint orange and yellow.

Ariadne's progeny in June are not *ariadne,* but a large and brilliantly orange insect. Are *ariadne's* size and hue, therefore, not inheritable, but dependent wholly upon the environment? At first thought this would seem to be the fact, and this was evidently the view of the matter presenting itself to Punnett when he stated that such variations are not inherited. We have seen, however, that they are hereditary in the sense that this organism must react in this particular way under these particular conditions. Its inherited organization compels, determines, this reaction.

These seasonal variations are therefore transmissible, though they are by no means "independent of climatic and other conditions." May they, therefore, play no part in evolutionary change? We should not yet be dogmatic as to this. The underlying hereditary basis in all probability is as susceptible to mutation, to disturbances in the arrangement and nature of the chromosomal elements, as any other germ-plasm. It would seem by no means impossible that the alternating phenotypes of *A.*

levana-prorsa, for example, might in suitable diverse climates, subarctic and tropical, respectively, be fixed as separate species.

A. levana bred in Labrador, for example, where it could produce only one brood, probably would not show its *prorsa*-producing tendency at all. This supposition is confirmed by Trybom's observation (quoted by Weismann) that in Siberia, where a single brood occurs yearly, it is *levana only.* Conversely, *prorsa* in the tropics would perhaps eliminate all traces of *levana,* though of this we can not be so confident.

How much practically identical germ-plasm in different parts of the world is masquerading as different species, because of the diverse ways in which it reacts to different environments in which it happens to be placed, has not been adequately investigated. An interesting example of the sort among tropical reef fishes was recently cited by Longley.[7]

> **_Bodianus fulvus_ and _B. punctatus_ are two color phases of one species of which one may almost instantaneously replace the other.**

The rapid interchange of reproductive habits between *Salamandra maculosa* and the alpine *atra* when transferred respectively to lowland or highland conditions, as described by Kammerer,[8] is probably also a case in point, due to fundamentally similar germ-plasm in both forms. Such an assumption would account for the inheritance of these readily acquired characters. If the facts are correct, *S. maculosa,* by cold and drought, was forced to assume the reproductive habits of the salamander of the neighboring alpine regions named *atra,* producing two adult larvæ viviparously, rather than many (14–72) immature embryos laid in water as is its habit in the warm, moist lowlands. Conversely, the alpine form was forced by heat and an ample water supply to increase its fecundity from two to nine larvæ at a birth. In both cases the "acquired characters" were inherited, as we would expect them to be if the two kinds of salamanders, as regards reproductive mechanism at least, have an identical or similar genotype. The fact that the lowland form living at higher altitudes has fewer young, and that the alpine form in the lower regions of its range produces an abnormally large number (viz., four) also points to the same conclusion.

It is a possibility worth considering that somatic modification accompanied by little germinal change may partially explain

[7] Carnegie Institution of Washington, Year Book, No. 14, 1915, p. 209.

[8] *Archiv f. Entwicklungsmechanik,* 25, 1907.

the remarkable "mimicry-rings" of South America that have been so interestingly discussed by Punnett. In each of several great regions of that continent a characteristic color pattern is exhibited by unrelated species belonging to different genera and even to different families. The color pattern followed in Central America differs slightly from that adopted by members of the same genera in eastern Brazil, a single genus of Pierids only dropping out of the ring in the latter region. In western Brazil and the upper Amazons the pattern is somewhat more mottled and the ground color darker, but the same genera are represented almost without exception. Finally, in Ecuador, Peru and Bolivia the pattern common to the different genera is still darker and greatly simplified. The Pierids here have left the ring, and a *Papilio,* an *Acræa* and two species of the Satyrid genus *Pedaliodes* have entered it. The point to be emphasized, however, is that the same genera, *e. g., Heliconius, Mechanitis,* have representatives in each local color group.

Let us now assume, with Punnett, that a set of similar or identical color factors is common to all the structurally diverse members of each ring, and add the further hypothesis that the color pattern resulting from these particular factors is to a large extent influenced by climatic conditions, as in seasonally polymorphic insects. It then follows that certain members of a "ring" migrating from the tropical climate of Brazil to the temperate zone farther south, even before they should become changed genotypically, would react to the new environment by assuming a new color pattern such as that now characteristic of the south temperate zone. If the genotype were identical throughout the migrating group and a single member of the group should so react, all naturally would respond in the same manner. Seasonal polymorphism thus may furnish an additional clue to the explanation of this most interesting case of convergence and parallelism in evolution.

This discussion leads to the conclusion that seasonal variations have a hereditary basis more sensitive than that of other color characters to temperature and other climatic conditions. A seasonal variation that is constant in its recurrence is transmissible. Its hereditary basis invariably reacts in a certain definite way to a certain narrow range of external conditions, whereas the hereditary basis of other characters, *e. g.,* eye color in vertebrates,

reacts in a precise way to a far wider range of external conditions.

Seasonal variations, as was pointed out by Weismann, show a hereditary tendency to alternate which, in some cases, is independent of external conditions.

Seasonal varieties are in some cases (*e. g.*, *Colias,* and possibly *Araschnia*) to be regarded as distinct ontogenetic stages. Cold arrests development at an early phase in color metabolism, and the mature insect emerges with pale colors (*Colias eurytheme* var. *ariadne*), or with a color pattern different from the definitive coloration of the species (*Araschnia levana*).

The suggestion is made that local color varieties, passing it may be for distinct species, are probably in some cases the equivalents of seasonal variations. That is, they are the product of a genotype sensitive to environmental changes expressing itself under a particular set of local climatic conditions; elsewhere the same genotype may respond quite differently. Such phenomena, though not of profound evolutionary significance, may play a rather conspicuous rôle in the evolution and diversification of the colors of animals and plants.

JOHN H. GEROULD

VARIATIONS IN THE VERMILION-SPOTTED NEWT, D. VIRIDESCENS

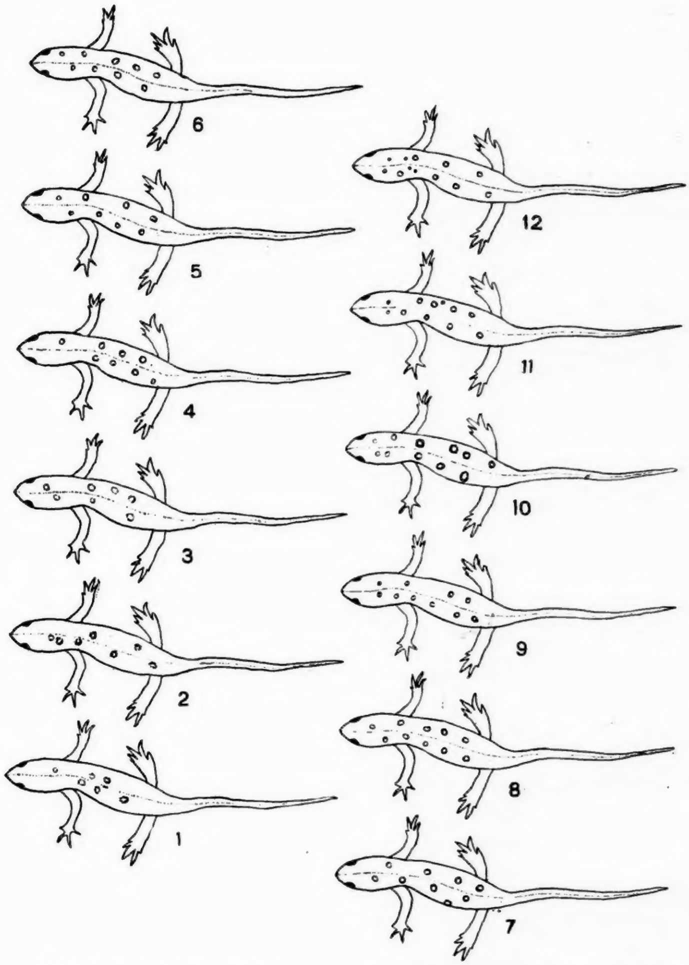
WHILE carrying on some experiments with the spotted newt, *Diemyctylus viridescens,* I was struck with the variation in the size, number and arrangement of the black-bordered vermilion spots so characteristic of this beautiful little salamander.

It is now generally recognized that this species exhibits two phases which were formerly described as distinct varieties or even species. As described by Gage[1] the young animal, which is terrestrial in habits, is red in color and was formerly called *D. miniatus;* later it becomes aquatic and its ground-color becomes olivaceous—permanently so, according to Gage. Against this dark ground-color (which is subject to considerable variation under different conditions even in the same individual) the bright red spots with their black borders stand out very strikingly.

It was with the olivaceous phase that I was experimenting, and it is upon this phase that the following observations are based.

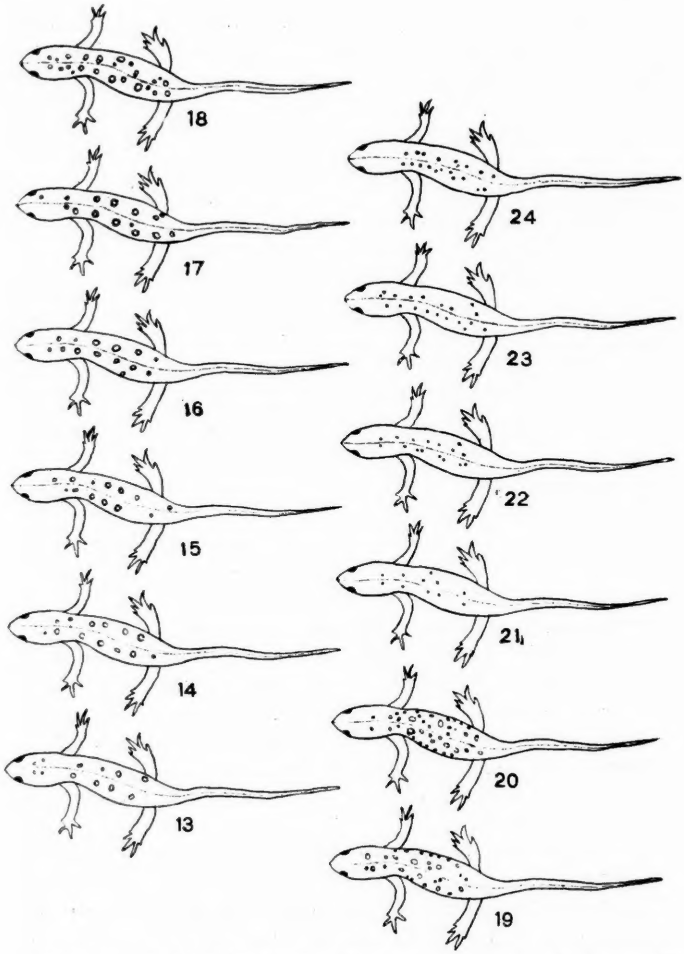
[1] Gage, S. H., "The Life-History of the Vermilion-Spotted Newt," AMER. NAT., December, 1891, pp. 1084–1103.

All the drawings were made from preserved material in which the vermilion spots had mostly faded to a white or pale pink color.



The first twenty figures were made from about three dozen specimens, probably all from the neighborhood of Morgantown. The last four figures are from animals that had been obtained from the Marine Biological Supply Company, Woods Hole, Mass.,

and had died, from time to time, in the laboratory aquaria. The mid-dorsal ridge is indicated in the figures by the dotted line.



Only the black-bordered vermilion spots were noted, the small black spots being too numerous and irregular to make it worth while to study them.

It will be noticed that in the animals from Woods Hole, shown in figures 21 to 24, the red spots were much smaller than most of

those on the animals from Morgantown. This was true of nearly but not quite all of the animals obtained from the north.

Cope says:[2]

> On each side of the vertebral line is a row of from three to six small round red spots, each with a black border. The rest of the surface is marked with small black points, which are smaller but more distinct on the lower surface.

Among all of the animals examined no two were spotted alike. They were sorted into groups according to the total number of red spots. The smallest number of red spots found was six; they were all of large size and arranged as shown in Fig. 1; only one animal with this number of spots was found.

Four animals were found that had seven red spots; Figs. 2 and 3 show the arrangement of the spots on two of these animals; all of the spots were large and of about the same size.

Four animals exhibited eight red spots, mostly large and of uniform size; two arrangements are shown in Figs. 4 and 5.

Three animals had nine red spots each, mostly large and of uniform size; Figs. 6 and 7 show two arrangements of these spots.

Seven animals had ten red spots each, this being the largest number of animals found in any group. The spots were mostly large and uniform in size; two arrangements are shown in Figs. 8 and 9. It will be noticed that in Fig. 8 the spots are arranged in fairly regular pairs.

Five animals had eleven red spots of somewhat more variable size than in the preceding. Figs. 10 and 11 show two arrangements of these spots; and Fig. 10, especially, shows wide variations in the size of the spots.

Three animals exhibited twelve red spots of variable size, two arrangements of which are shown in Figs. 12 and 13.

Two animals, shown in Figs. 14 and 15, exhibited thirteen red spots of various sizes.

Two animals had fourteen red spots; one of these animals is shown in Fig. 16.

Figs. 17, 18, 19 and 20 show the arrangements of red spots on four animals that had 15, 24, 29 and 39 spots, respectively. It will be noticed in these animals, especially in the last, that the large number of red spots is due to an increase in the number of very small spots, the number of large red spots being no greater than in the earlier individuals. Thirty-nine was the largest num-

[2] Cope, E. D., "The Batrachia of North America," *Bull. U. S. Nat. Mus.*, No. 34, 1889, p. 210.

ber of red spots found on any single animal. Only one animal in each of these last five groups was found.

Figs. 21 to 24, as noted above, represent animals obtained from Woods Hole; they have 11, 16, 19 and 20 spots, respectively, and it will be noted that all of the spots are small and of fairly uniform size.

Conclusion

It would seem from this hurried survey that the number, size and arrangement of the vermilion spots, so characteristic of *D. viridescens,* are quite variable, probably two animals very seldom being even approximately alike.

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